

CUK-400503-5-122373

JOURNAL
• OF THE
INDIAN CHEMICAL SOCIETY



VOLUME XVI

Title Page, Contents, Indexes and Errata.

1939

CALCUTTA UNIVERSITY PRESS

• 122373

122373.

122373

JOURNAL
of the
INDIAN CHEMICAL SOCIETY
Volume XVI, 1939.

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ERRATA:

Page.	Line.	Read.	for.
67	8	being	is
	Formula I	$\text{HO}_2\text{C}-\text{CH}=\overset{\text{CHMe}_2}{\text{C}}-\text{CH}_2\cdot\text{CO}_2\text{H}$	
	„ (II)	$\text{H}_2\text{C} \begin{array}{l} \text{CHMe}_2 \\ \\ \text{C} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H} \\ \\ \text{CH} \cdot \text{CO}_2\text{H} \end{array}$	
69	7*	13 g.	1·3 g.
100	Caption	Part III.	Part II.
107	5*	1935	1835
108	12	(VI, R = c. h.)	(VI, R = c. p.)
126	8	Carbonyl	Carboxyl
	1*	thioindogenide	thioindigenide
128	11	dyeing	dying
129	11	violet	voilet
308	Caption	A note...solutions of alkali on potassium ferricyanide	A note...solutions of potassium etc.
371	Eq. (1) as	$\frac{\eta - \eta_0}{\eta_0} = A \sqrt{\frac{1}{a} t} + \beta (1 - a) c$	
373	Table I	$k = 2 \cdot 14 \times 10^{-4}$	$k = 2 \cdot 4 \times 10^{-4}$
677	8	$\begin{array}{c} \text{H}_3\text{CO} \\ \text{Cl} \end{array} \text{C}_6\text{H}_3 \cdot \text{CONH} \cdot \text{CH}(\text{Cl}) \cdot \text{CCl}_3$	

* From bottom.

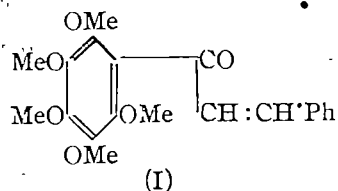
THE CONSTITUENTS OF *DIDYMOCARPUS PEDICELLATA*. PART II. COMPARATIVE STUDIES IN THE CONSTITUTION OF PEDICIN, ISOPEDICIN, PEDICININ, AND PEDICELLIN.

BY VISHWANATH SHARMA AND SALIMUZZAMAN SIDDIQUI.

Investigations in the constitution of the colouring matters of *Didymocarpus Pedicellata*, reported in the earlier communication, were carried out chiefly by studying their behaviour towards caustic soda, permanganate, nitric acid and bromine. As a result of these studies they have been established as derivatives of chalcone and benzalcoumaranone. Pedicellin has been shown to be 2:3:4:5:6-pentamethoxychalcone, pedicin to be 5:6-dihydroxy-2:3:4-trimethoxychalcone, pedicinin to be 3:5:6-trihydroxy-4-methoxybenzalcoumaranone and isopedicin to be 5-hydroxy-2:3:4-trimethoxy-benzylcoumaranone. Incidentally it has been brought out that these colouring matters are not allied to dunnine as suggested by Robinson in a recent note on the colouring matter of *Streptocarpus Dunnii*, Mast.

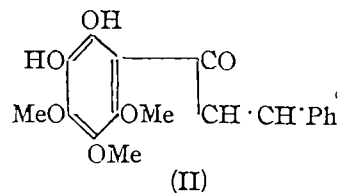
The preceding communication (Siddiqui, *J Indian Chem. Soc.*, 1937, 12 705) dealt with the isolation and characterisation of the principal colouring matters of *Didymocarpus pedicellata*, namely pedicin, $[C_{18}H_{18}O_6 : C_{14}H_7(CO)(OH)_2(OMe)_3]$ and the three apparently allied products, isopedicin ($C_{18}H_{18}O_6$), pedicinin $[C_{16}H_{12}O_6 : C_{15}H_8O_4(OH)(OMe)]$ and pedicellin $[C_{20}H_{22}O_6 : C_{15}H_7O(OMe)_5]$, the position arrived at in regard to their chemical constitution and mutual relationship being expressed in their simplified formulae above. Their colours and colour reactions with concentrated sulphuric acid and caustic soda indicate that they do not have a flavone or an isoflavone structure and might be allied to chalcones, while the ferric chloride colour reaction of pedicin suggests that its two hydroxyls are in *ortho*-position.

The present paper embodies the results of subsequent investigations which were principally based on studies in the action of caustic soda, permanganate, nitric acid and bromine. These investigations fairly establish the products under discussion to be derivatives of chalcone and benzalcommarone in which the position of the substituents may be indicated as shown in the following tentative structures:



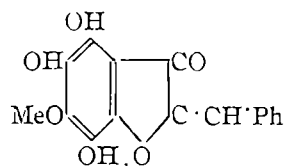
Pedicellin

(2:3:4:5:6-Pentamethoxychalcone),



Pedicin

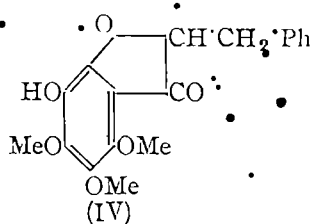
(5:6-Dihydroxy-2:3:4-trimethoxychalcone),



(III)

Pedicinin

(3,5,6-Trihydroxy-4-methoxy-benzalcoumaranone).



(IV)

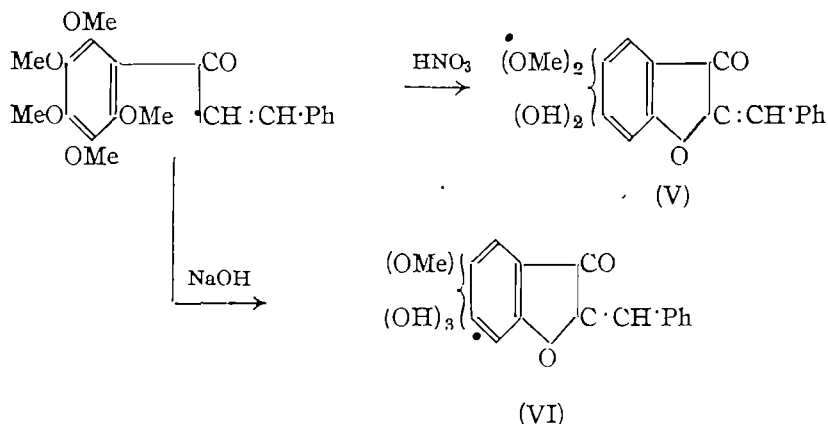
IsoPedicin

(5-Hydroxy-2,3,4-trimethoxy-benzyl-coumaranone)

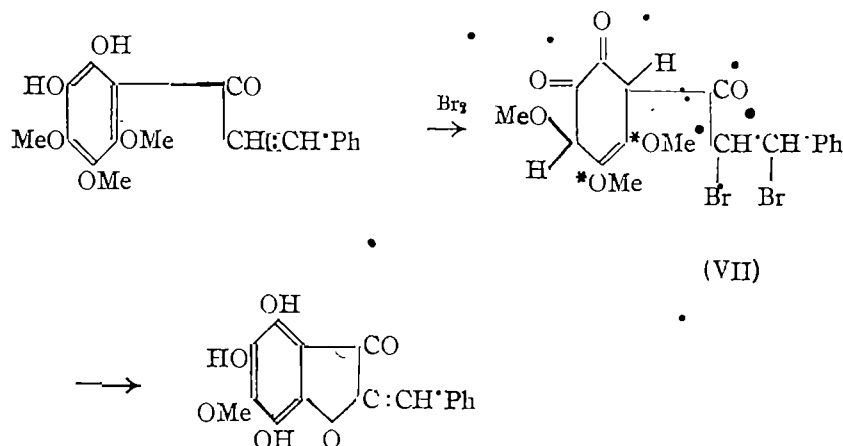
Pedicellin is established as a dimethyl ether of pedicin because (a) pedicin is converted to pedicellin on methylation with dimethyl sulphate and the demethoxylation of both these products gives the same pentahydroxy compound which has been named des pedicellin; (b) the phenylhydrazone of pedicellin indicates the presence in it of a keto group which was already noted in pedicin (Part I, *loc. cit.*); and (c) the formation of dibromo derivatives of pedicellin and dibenzoylpedicin indicate the presence of a single double bond in each one of them. As on heating with caustic soda solution, and also on oxidation with hydrogen peroxide or permanganate, both pedicin and pedicellin yield benzaldehyde, apart from an uncrystallisable acid, which is still under investigation, the presence of a $C_6H_5CH:C<$ grouping in them is considered plausible. These observations simplify the formulae of pedicin and pedicellin to $C_6H(OH)_2(OMe)_3(CO)(C=CH\cdot Ph)$ and $C_6H\cdot(OMe)_3(CO)(C=CH\cdot Ph)$ respectively. It is further noted, that the pale yellow isopedicin, which is saturated to bromine, is converted into the unsaturated, orange-red pedicin by caustic soda at the ordinary temperature. This is apparently due to the rupture of a ring. The isomerisation is not due to the enolisation as isopedicin could not be retransformed to pedicin. The relationship between pedicin, isopedicin and pedicellin is thus cleared up.

The relationship of these products to pedicinin is indicated by a series of reactions which simultaneously lead to the elucidation of their constitution. On treatment of pedicellin in glacial acetic acid solution with concentrated nitric acid, it yields a light orange coloured dehydro product, which contains only two methoxyls as against five in pedicinin. This proves to be monomethoxypedicinin, as it undergoes demethylation with dilute alkali at the ordinary temperature and is converted into a product identified as pedicinin. The colour reactions of pedicinin and the fact that benzaldehyde is produced when it is treated with alkali exclude a flavone or isoflavone structure for it. Again, since pedicinin contains the

same number of oxygen atoms as pedicellin, it appears possible to explain these reactions only by extending the simplified formula of pedicellin arrived at above, to (I), in which all the methoxyls are substituted in the same benzene ring. The action of nitric acid and caustic soda may then be represented as



The constitution of pedicin as represented by (II) follows on the one hand from its relation to isopedicin, which indicates the *ortho-meta* position for its two *ortho* hydroxyls, and on the other from its relationship to pedicellin which is its dimethyl derivative. Further, on treatment with two atoms of bromine in chloroform solution in the cold, pedicin yields pedicinin as a result of the cumaranone condensation of the initially formed dibromo derivative and the simultaneous removal of 2 out of its 3 methoxyls. The removal of these two methoxyl groups under such mild conditions could be explained by assuming that the desmotropic modification of the two *o-m*-hydroxyls makes the methoxyls marked * in formula VII, situated on a single double bond in the ring, exceptionally unstable and a similar explanation may also account for the exceptional instability of the *o*-methyl in methylpedicinin, noted above. The position of the methoxyl group in pedicinin has been based on this assumption, and this gets further support from its colour reaction with ferric chloride indicating the unsymmetrical position of its 3 hydroxyls. The action of bromine on pedicin which ultimately results in the elimination of two CH_3Br may be represented in the following manner:



The above explanation finds further support from the fact that when this desmotropy is guarded against by benzoylating or methylating pedicin, the action of bromine does not result in the formation of dibenzoyl or dimethylpedicin but yields in the normal way dibromodibenzoylpedicin and dibromopedicellin respectively. The presence of a double bond in pedicin is also established by the formation of these two derivatives, as well as by its catalytic reduction with platinum black to a dihydro derivative.

The oxidation of pedicin with permanganate also leads to pedicinin in an apparently analogous manner. Along with the latter, benzaldehyde (characterised by phenylhydrazone, semicarbazone and conversion to benzoic acid) is formed due to hydrolysis of pedicinin.

The demethylation of pedicinin gives a product whose analysis, melting point and mixed melting point show it to be identical with despedicellin. This is obviously due to the rupture of the oxygen ring in the process of demethylation with hydroiodic acid. Despedicellin as obtained from any of the three products (I, II or III) has a pale yellow colour, absorbs bromine in cold, gives a deep red colouration with concentrated sulphuric acid, and a dark bluish green colour and smell of benzaldehyde with alkali. With ferric chloride it gives a dark bluish green colouration changing through light orange-red to reddish violet. On the other hand, it gives no characteristic colour of flavones with Mg and HCl. Its constitution as 2:3:4:5:6-pentahydroxychalcone may thus be regarded as fairly established.

The occurrence of pentahydroxybenzene derivatives in nature can not be considered improbable as various authors have in more recent years proved their presence in a number of plant families. Calycoptin from *Calycopteris Floribunda* (Ratnagiriswaran, Sehra and Venkataraman, *Biochem. J.*, 1934, 28, 1964) and the identical flavone thapsin from

Digitalis thapsi (Karrer, *Chem. Zentr.*, 1935, I, 715) and also robilitin from *Citrus nobilis* (Tseng and Tseng and Robinson, *J. Chem. Soc.*, 1938, 1009) may be given as instances. On the other hand, the occurrence of a number of pentahydroxybenzene derivatives belonging to the chalkone and benzalcoumaranone series is of interest. Attempts to synthesise these products are engaging our attention.

Robinson (*Nature*, July, 1938) has recently communicated the isolation of a colouring matter, dunione, $C_{15}H_{14}O_3$, from the deposit on the leaves and inflorescence of *Streptocarpus Dunn*, Mast, and has in view of the close generic relationship of this plant to *Didymocarpus pedicellata* suggested the likelihood of its being related to the colouring matters of the pedicin series. The work embodied in the present paper does not, however, support this suggestion as these products for which a chalkone structure was suggested in the earlier communication have now been definitely established as chalkone and benzalcoumaranone derivatives, while a 2:3:3-trimethyl-6:7-benzocoumaranone-4:5-quinone or its gem-dimethyl isomeride structure has been suggested for dunione.

EXPERIMENTAL.

Methylation of Pedicin : Pedicellin.—To a solution of pedicin (0.4 g.) dissolved in 10 c.c. of 15 % sodium hydroxide solution, dimethyl sulphate (3 c.c.) was slowly added with shaking. The methylated product separated out after some time and on crystallisation from ether yielded colourless stout rods which melted at 98° and gave no depression on admixture with a pure sample of pedicellin, yield 0.35 g. (Found after drying to constant wt. at 50° in *vacuo* over P_2O_5 : C, 67.09; H, 6.14. $C_{20}H_{22}O_6$ requires C, 67.03; H, 6.13 per cent).

Demethylation of Pedicellin. Des-pedicellin.—Pedicellin (3.5 g.) was refluxed with HI (d 1.77, 10 c.c.) for about 4 hours in a metal bath at 130° - 40° . The resultant product was poured into water and the brick red insoluble powder was filtered, washed with water and thio-sulphate solution and dried on a porous plate. The crude demethylated product was successively crystallised from acetone, ethyl acetate and methanol when it finally melted at 255 - 56° (decomp.) with shrinking and partial sublimation at 230° , yield 2.3 g. Despedicellin, thus obtained, formed pale yellow glistening silky needles insoluble in petrol ether, sparingly soluble in ether but fairly soluble in acetone, alcohol or hot ethyl acetate. It is soluble in caustic soda with a dark bluish green colour and begins to decompose in alkaline solution on keeping, giving smell of benzaldehyde. It could not be methylated with dimethyl sulphate

in the usual way. (Found after drying to constant wt. at 100° in *vacuo* over P_2O_5 : C, 62.5; H, 4.3. $C_{15}H_{12}O_8$ requires C, 62.5; H, 4.2 per cent). In alcoholic solution it gives with ferric chloride a deep bluish green colour which fades through light orange-red to reddish violet. On addition of a further drop of ferric chloride it develops a light brownish red colour.

Demethylation of Pedicin and Pedicinin: Despedicellin.—The demethylation products of pedicin, pedicinin and methylpedicin were obtained in a similar manner as in case of pedicellin and were found to be identical with despedicellin by their m. p. and mixed m. p., formation of similar phenylhydrazones, colour reactions and analyses. [Found after drying demethoxy-pedicin to constant wt. at 100° in *vacuo* over P_2O_5 : C, 62.6; H, 4.3. Demethoxy-pedicin gave after drying: C, 62.5; 62.2; H, 4.2; 4.3; demethoxy-methylpedicin gave C, 62.4; H, 4.1. $C_{15}H_{12}O_8$ requires C, 62.5; H, 4.2. $C_{15}H_{10}O_8$ requires C, 62.9; H, 3.5 per cent).

Despedicellin-phenylhydrazone.—Despedicellin (0.3 g.) was heated in an alcoholic solution with phenylhydrazine (0.2 c.c.) and a drop of acetic acid on a water-bath for $\frac{1}{2}$ hour. The dark red solution was poured into very dilute acetic acid and the precipitated red sticky mass was washed with water and dissolved in ethyl acetate. On concentration and cooling the solution it yielded long colourless needles of despedicellin-phenylhydrazone, m.p. 196° . (Found after drying to constant wt at 50° in *vacuo* over P_2O_5 : C, 66.66; H, 5.4. $C_{15}H_{12}O_8 \cdot C_6H_5N_2$ requires C, 66.66; H, 4.7 per cent).

The phenylhydrazones of demethylated pedicin, pedicinin and methylpedicin, prepared in an analogous manner, melted at 196° , 197° and 195° respectively and showed no depression in m.p. on admixture with each other or with despedicellin-phenylhydrazone.

Treatment of Pedicin with Bromine: Pedicinin and Dibromopedicin.—Pedicin (0.1903 g.) was kept with 0.1 g. of bromine (2 atoms) in chloroform solution for a few hours in cold. The bromine was found to be in excess on testing with starch-iodide paper. The solvent was evaporated off under a fan and the residue was dissolved in methanol. On concentration of the methanol solution, carmine red crystalline aggregates of stout rods were obtained, which were free from bromine, melted at 203° and were found to be identical with pedicinin. (Found: C, 63.8; H, 4.1. $C_{16}H_{12}O_8$ requires C, 64.0; H, 4.10 per cent). The mother-liquor on concentration yielded another crop of pedicinin (total 0.07 g., ca 40%). The final filtrate appeared to contain yet another bromine-free product which is under investigation.

On keeping pedicin with 4 equivalents of bromine in the cold, a reddish brown crystalline product was obtained, m.p. 180° (decomp.). (Found after drying at 100° in *vacuo* over P_2O_5 : Br, 35.3. $C_{16}H_{12}O_6Br_2$ requires Br, 34.8 per cent).

Bromination of Benzoylpedicin: Dibromodibenzoylpedicin.—Benzoylpedicin (0.1836 g., m.p. 181°) was kept with 0.1054 g. of bromine (equivalent to 4 Br) in chloroform solution in cold for a few hours. The residue obtained after evaporation of the solvent gave 0.14 g. of a dibromo derivative which crystallised from methanol in colourless stout rods, m.p. 170° (decomp.). [Found after drying to constant wt. at 50° in *vacuo* over P_2O_5 : Br, 22.5. $C_{18}H_{16}O_6 (C_6H_5CO)_2 Br_2$ requires Br, 22.9 per cent].

Bromination of Pedicellin Dibromopedicellin.—To a chloroform solution of pedicellin (1 g.) was added a chloroform solution of bromine (equivalent to 2 Br) at the ordinary temperature and the solvent was completely removed on the water-bath. On cooling the petrol ether solution of the residue, the bromo derivative crystallised in rods and needles m.p. 132° . It was finally crystallised from a mixture of ethyl acetate and ether (in which it is more readily soluble than in petroleum ether) when it came out in bunches and stars of stout rods and needles, yield 1.2 g. (Found in air-dried sample. Br, 30.3. $C_{20}H_{22}O_6Br_2$ requires Br, 30.5 per cent).

Pedicellin-phenylhydrazine.—Pedicellin (0.3 g.) was warmed on a water-bath with phenylhydrazine (0.2 c.c.) with a drop of acetic acid for $\frac{1}{2}$ hour. The sticky mass, obtained by pouring the solution into very dilute acetic acid, was washed with water, taken up in ethyl acetate, concentrated to a small volume, and cooled, when the phenylhydrazone crystallised out in colourless rods, m.p. $133-35^{\circ}$. (Found after drying to constant wt. at 50° in *vacuo* over P_2O_5 : C, 69.5; H, 6.30; N, 6.35. $C_{26}H_{28}O_5N_2$ requires C, 69.6; H, 6.25; N, 6.25 per cent).

Action of Nitric Acid on Pedicellin (98°): Methylpedicinin, $C_{17}H_{14}O_6$.—Pedicellin (1 g.) in glacial acetic acid (2.5 c.c.) was slowly treated with concentrated nitric acid (d 1.4, 0.5 c.c.) and the solution was immediately diluted with water, when an orange yellow oily product separated out which solidified on cooling. The supernatant liquid was shaken out with ethyl acetate and the solidified product was dissolved in the ethyl acetate extract. The solution was well washed with water, dried with sodium sulphate, concentrated to a small volume and cooled, when a yellowish crystalline mass separated out. Methylpedicinin, thus obtained, melted at 110° , yield 0.52 g. The melting point remained unaltered on crystallisation from alcohol but the colour appeared to be light orange. It is

fairly soluble in ethyl acetate and alcohol, less so in ether and nearly insoluble in petroleum ether. It dissolves in sulphuric acid with a blood red colour which later changes to reddish brown. [Found after drying to constant wt. in *vacuo* over P_2O_5 : C, 65.1; H, 4.5; OMe, 18.4. $C_{17}H_{14}O_6$ requires C, 65.0; H, 4.4; OMe, 19.8 per cent].

On dissolving methylpedicin in dilute sodium hydroxide solution and acidifying the alkaline solution with dilute hydrochloric acid a dark red product was obtained which on crystallisation from chloroform yielded aggregates of carmine red rods and needles, m.p. 203° . It showed no depression on admixture with a pure sample of pedicin. (Found: C, 63.8; H, 4.0. $C_{16}H_{12}O_6$ requires C, 64.0; H, 4.0 per cent).

Conversion of isoPedicin to Pedicin.—isoPedicin (m.p. 105° , 0.1 g.) was dissolved in 30% sodium hydroxide solution and immediately acidified with hydrochloric acid. On crystallisation of the precipitate from ether pedicin was obtained in orange coloured rectangular plates, m.p. 143° and giving no depression on admixture with pure pedicin.

Oxidation of Pedicin with Potassium Permanganate.—Pedicin (0.43 g.) was dissolved in very dilute sodium hydroxide solution and a 5% solution of potassium permanganate (0.36 g. equivalent to 2O) was slowly added to the alkaline solution with ice cooling. The reaction mixture was separated from the precipitated manganese dioxide and extracted with ether. The ethereal solution yielded an oily residue smelling of benzaldehyde. It was oxidised to benzoic acid, and its phenylhydrazone and semicarbazone which melted at $159-61^\circ$ and 221° respectively showed no depression on admixture with authentic samples.

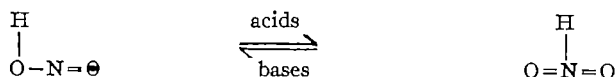
The alkaline solution was acidified with hydrochloric acid and the precipitate crystallised from chloroform, when it yielded bunches and stars of carmine red rods and needles, m.p. 203° and showed no depression in m.p. on admixture with pedicin. (Found after drying to constant wt. at 100° in *vacuo* over P_2O_5 : C, 63.8; H, 4.0. $C_{16}H_{12}O_6$ requires C, 64.0; H, 4.0 per cent).

Reduction of Pedicin.—Pedicin (1 g.) was shaken with platinum black (0.1 g.) in methyl alcoholic solution for 9 hours during which 70 c.c. of hydrogen were absorbed (required for 1 mol., 65 c.c.) The yellow solution was filtered, freed of the solvent and the oily residue crystallised from ether when it yielded pale yellow hexagonal plates, m.p. $120-21^\circ$, yield 0.8 g. (Found after drying to constant wt. at 50° in *vacuo* over P_2O_5 : C, 64.7; H, 6.0. $C_{18}H_{20}O_6$ requires C, 65.0; H, 6.0 per cent).

THE TAUTOMERISM OF NITROUS ACID.

By H. KRALL.

A tautomeric constitution for nitrous acid :



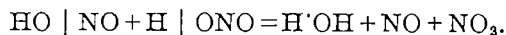
explains many otherwise obscure observations. The *hydroxy* form predominates. Mineral acids favour the *nitro* form and the change proceeds rapidly, the reverse change is slow. It is the *nitro* form which acts as an oxidising agent.

Laar (*Ber.*, 1885, **18**, 655) suggested the tautomerism of nitrous acid and in view of the importance of this reagent, the re-examination of the subject seems overdue.

Nitrous acid has been extensively used by Werner in his investigations of carbamide and thiocarbamide (*J. Chem. Soc.*, 1917, 111, 863, 1912, **101**, 2180) on the assumptions that (i) nitrous acid reacts quantitatively with an amino group involving nitrogen, and (ii) if any alteration in the experimental conditions (usually p_{H}) alters the amount of nitrogen involved, this may be taken both as an indication and as a measure of tautomeric change in the substance under examination. The same rationale has been used by the present author in the study of phenylthiocarbamide and its homologues (Krall *et al.*, *J. Indian Chem. Soc.*, 1935, **12**, 640; 1937, **14**, 478, 1938 **15**, 217).

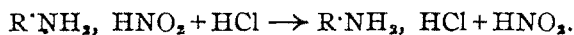
If nitrous acid itself is tautomeric and if, as appears to be the case, its equilibrium is displaced by change of p_{H} , this does not necessarily invalidate the inferences previously drawn, but clearly imposes caution owing to the recognition of an unsuspected factor.

Now if nitrous acid is not tautomeric, its spontaneous decomposition must occur according to some such process as



But to the present author it appears axiomatic that under conditions of molecular homogeneity (*e.g.* in the absence of a 'reagent') one molecule can break down in only one way; and whenever a *compound* breaks down in two different ways simultaneously this fact indicates the existence of two different *molecules*, *i.e.* tautomerism. This principle is not sufficiently widely

the displacement $\text{HO}\cdot\text{NO} \rightarrow \text{H}\cdot\text{NO}_2$ is more strongly influenced by mineral acids than the displacement

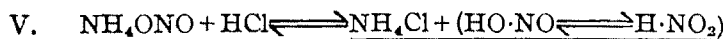
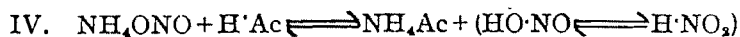


The main difference between Taylor's results for the amino-acids and for methylamine is that while the reaction is termolecular in both cases, it does not set in at all with methylamine with only one equivalent of nitrous acid; but this is not surprising, for methylamine is a strong enough base to hold up a whole equivalent; there *need* only be enough 'salt formation' to provide the cation $\text{R}\cdot\text{NH}_3$, since it is this which actually interacts (Werner, *loc. cit.*, p. 865; Taylor, *loc. cit.*, p. 1905). The stability of the salts of methylamine no doubt greatly facilitates investigation by physical methods, but the results cannot be regarded as typical since amines are usually weak bases; results obtained with amino-acids are clearly better worth close investigation.

As Taylor's procedure was directed to measuring the rates rather than the results of reaction, his experiments are not comparable with those obtained in the nitrometer in the study of carbamides and thiocarbamides. In the following experiments we followed the latter method (*loc. cit.*) using milli-molar quantities of the reagents named and allowing the reactions to proceed for 24 hours. The total quantity of gas obtainable if the reaction went to completion would be 11 c.c. Brown oxides of nitrogen are not obtained as they are reduced by the mercury to nitric oxide.

	N_2	NO and NO_2
I. Ammonium nitrite gave 1.16 c.c. solely N_2 ,	say 1	0
II. Sodium nitrite and acetic acid gave 0.8 c.c. solely NO,	0	1
III. Sodium nitrite and hydrochloric acid gave 6.6 c.c. solely NO,	0	7
IV. Ammonium nitrite and acetic acid gave 7.1 c.c. in which NO is 13%,	6	1
V. Ammonium nitrite and hydrochloric acid gave 7.2 c.c. in which NO is 71%,	2	5

In IV and V the acids will compete for the base, so we may write:



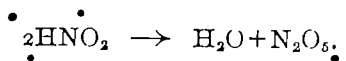
the predominant components being underlined. Now (from I) ammonium nitrite alone only gave 1 c.c. of nitrogen, therefore the left side of IV must have given not more than 1 c.c. but the whole of IV gave 6 c.c., therefore

the *right* side (progressively replenished, of course) gave 5 c.c. ; in other words it is nitrous acid and the cation which interact as maintained by both Werner and Taylor. Probably the decomposition of ammonium nitrite alone takes place in the same way, the nitrous acid being furnished by hydrolysis



Further, if IV is compared with V, it will be evident that since the right side of V represents the predominant condition it should be expected to give more than 5 c.c. but in fact the whole change only furnishes 2 c.c. That is to say that although the right side of V provides more of the ammonium anion and also more free nitrous acid than IV, the nitrogen produced is less ; evidently the nitrous acid is not available to such an extent in V and this is at once explained if it is tautomeric and if mineral acids favour the form $\text{H}'\text{NO}_2$. Indeed the figures for IV, and V, in themselves at once suggest some change of constitution in the reactants rather than a p_H effect of acceleration or retardation.

The alternative view that the text-book decomposition of nitrous acid as retarded by mineral acids sufficiently explains these facts can no longer be accepted for the following reasons : (i) As already pointed out the usual formulation of the decomposition of nitrous acid is itself very hard to accept, and is only one degree less absurd than the view that dehydration takes place even in dilute solution .



(ii) It is not clear why hydrogen ions should influence any of these changes, whereas on the tautomeric hypothesis, an effect of hydrogen ions would be anticipated and is in fact observed (iii) The difference between II and III (noting that excess of acid was not used, but only one equivalent, sufficient to liberate the nitrous acid) cannot be due to any *catalytic* effect of hydrogen ions, but indicates clearly that the nitrous acid liberated in one case is not the same as that liberated in the other ; if the 'decomposition' of nitrous acid is an interaction between two tautomers, it will be greatest when they are present in equal amounts, and the decomposition will, therefore, be 'accelerated' or 'retarded' by mineral acids according to the conditions of experiment.

To ascertain the equilibrium between the tautomers would necessitate careful and perhaps difficult dynamic experiments, but a guess may be hazarded that in the absence of acids, stronger than itself, there is less than

10% of the form $\text{H}\cdot\text{N}\text{O}_2$ (II and IV), and that when liberated by one equivalent of hydrochloric acid under above conditions this form increases to some 30% of the whole.

The above view of nitrous acid affords a simple explanation of the results obtained by Macmillan and Reade (*J. Chem. Soc.*, 1929, 2863) and Donald and Reade (*ibid.*, 1935, 53) who found that certain aromatic tertiary amines gave simultaneously (a) nitro compounds and (b) nitrosoamines and that high temperatures and high acidity favoured (a), whereas the contrary conditions favoured (b). There could hardly be clearer proof of the views expressed above.

EXPERIMENTAL.

Molar solutions of ammonium chloride, sodium nitrite, hydrochloric acid and acetic acid, all of A. R. quality, were prepared in distilled water. All the experiments were carried out in a Lunge nitrometer in the same way as urea and thiocarbamide experiments (*loc. cit.*). The reaction mixture was in all cases 3 c. c. and was not shaken. The volume of gas was noted after 24 hours. Concordant results were obtained.

I. *Decomposition of aqueous ammonium nitrite.*—Sodium nitrite ($\frac{1}{2}$ c. c.) and ammonium chloride ($\frac{1}{2}$ c. c.) were introduced and washed down with water (2 c. c.). Gas evolved 1.4 c. c. at 31° and 736 mm. or 1.16 c. c. at N.T.P. and was wholly nitrogen.

II. *Decomposition of nitrous acid liberated by one equivalent of acetic acid.*—Sodium nitrite ($\frac{1}{2}$ c. c.) was introduced and washed down with water ($\frac{1}{2}$ c. c.); acetic acid was then added ($\frac{1}{2}$ c. c.) and washed down with water (1 c. c.). Only minute bubbles were observed and the mercury was not appreciably tarnished. Gas evolved 1 c. c. at 29° and 735 mm. or 0.8 c. c. at N.T.P. and was wholly nitric oxide.

III. *Decomposition of nitrous acid liberated by one equivalent of hydrochloric acid.*—As in II, but using hydrochloric acid ($\frac{1}{2}$ c. c.) instead of acetic acid. The evolution of gas was more rapid than in II, and measured 7.9 c. c. at 30° and 735 mm. or 6.6 c. c. at N.T.P. and was wholly nitric oxide. Brown oxides of nitrogen were not obtained in this or any other case as they were reduced by the mercury which became tarnished.

IV. *Decomposition of ammonium nitrite in the presence of one equivalent of acetic acid.*—Sodium nitrite ($\frac{1}{2}$ c. c.) and ammonium chloride

($\frac{1}{2}$ c. c.) were introduced and the cup washed down with water ($\frac{1}{2}$ c. c.). Acetic acid ($\frac{1}{2}$ c. c.) was then added and washed down with water (1 c. c.). The reaction was slow. Gas evolved 8.7 c. c. at 33° and 736 mm. or 7.1 c. c. at N.T.P. and contained nitric oxide 13% and nitrogen 87%.

V. *Decomposition of ammonium nitrile in the presence of one equivalent of hydrochloric acid.*—As in IV, but using hydrochloric acid ($\frac{1}{2}$ c. c.) instead of acetic acid. The reaction was rapid at first and slow later; the mercury was tarnished. Gas evolved 8.8 c. c. at 32° and 736 mm. or 7.2 c. c. at N.T.P. and contained nitric oxide 71% and nitrogen 29%.

The author desires to acknowledge the help of Mr. K. Beharilal, M.Sc. in these experiments.

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Received December 23, 1938.

VARIATION OF THE CATAPHORETIC VELOCITY OF SILVER HALIDES IN PRESENCE OF DIFFERENT DYESTUFFS.

BY M. K. INDRA.

Measurements of cataphoretic velocities in presence of increasing concentrations of different dyestuffs have been made, which show that there is, in general, an increment of the $c. v$ values with increasing concentrations of the dye. There is also observed a time effect.

The theory of adsorption indicators as put forward by Fajans (*Z. physikal. Chem.*, 1931, 158, 107) or Kolthoff (*Chem Rev.*, 1935, 16, 87) has been shown to be not in good agreement with facts as observed by measurements of cataphoretic velocities under the exact conditions of titration (Chaudhury and Indra, to be published in the next issue of *this Journal*). Moreover these observations clearly indicate the specific nature of adsorption. In this paper measurements with increasing concentrations of dyestuffs have been made which clearly emphasize the specific nature of the adsorption of dyestuffs by silver halides.

EXPERIMENTAL.

Experiments were carried out with 1.8 c. c. of 0.1N-halide and 2 c. c. of 0.1N-silver nitrate with increasing amounts of the different acidic indicators. With methyl violet as indicator, 1.8 c. c. of 0.1N-silver nitrate and 2 c. c. of 0.1N-KCl were taken. The results are given in Tables I—VI.

TABLE. I

Silver iodide and eosin.

0.1N-KI = 1.8 c.c. 0.1N-AgNO₃ = 2 c.c. Total volume = 100 c. c.

Na-eosinate	Nature of the charge.	Time after mixing.	$V \times 10^5$.
0.3 mg.	-ve	4-8 min.	14.0
		20-24	15.5
0.6	-ve	5-10	11.0
		15-19	15.24
		26-29	16.36
0.9	-ve	6-10	18.1
		16-19	21.1
1.2	-ve	5-7	20.83
		15-19	22.9

* V in Tables I—VI indicates velocity in cm./sec. per volt./cm.

TABLE II.

*Silver iodide and fluorescein*0.1N-KI=1.8 c. c. 0.1N-AgNO₃=2 c. c. Total volume=100 c. c.

Fluoresceinate.	Nature of the charge.	Time after mixing.	$V \times 10^5$.
0.15 mg.	+ve	5-9 min.	16.32
		15-19	20.85
	-ve	after 3 hrs.	15.48
0.3	-ve	5-10 min.	18.04
0.6	-ve	5-10	23.6
1.2	-ve	6-10	24.63
2.4	-ve	5-10	25.05

TABLE III.

*Silver bromide and eosin*0.1N-KBr=1.8 c. c. 0.1N-AgNO₃=2 c. c. Total volume=100 c. c.

Na-eosinate	Nature of the charge	Time after mixing.	$V \times 10^5$.
0.3 mg.	+ve	7-11 min.	19.68
		27-30	22.88
		36-40	23.2
	-ve	after 3 hrs.	...
0.6	-ve	5-10 min.	17.46
1.2	-ve	4-7	17.5
		12-16	18.26
1.8	-ve	5-9	29.9
		19-23	24.83

TABLE IV.

Silver bromide and fluorescein.

0.1N-KBr=1.8 c.c. 0.1N-AgNO₃=2 c.c. Total volume=100 c.c.

Fluoresceinate	Nature of the charge.	Time after mixing.	$V \times 10^5$.
0.15 mg	+ve	4-8 min	17.6
	-ve	after 3 hours.	16.8
0.3	-ve	7-12 min.	19.9
		19-23	26.24
0.6	-ve	5-8	25.16
1.2	-ve	5-10	22.27
2.4	-ve	5-10	24.55
		16-20	27.75

TABLE V.

Silver chloride and fluorescein.

0.1N-KCl=1.8 c.c. 0.1N-AgNO₃=2 c.c. Total volume=100 c.c.

Na-fluoresceinate	Nature of the charge.	Time after mixing.	$V \times 10^5$.
0.15 mg.	-ve	5-10 min.	11.47
0.3	-ve	4-8	14.96
0.6	-ve	5-10	17.5
1.2	-ve	5-10	20.83

TABLE VI.

Silver chloride and methyl violet.

0.1N-KCl=2 c.c. 0.1N-AgNO₃=1.8 c.c. Total volume=100 c.c.

Na-salt of methyl violet.	Nature of the charge.	Time after mixing.	$V \times 10^5$
0.15 mg	-ve	4-8 min.	12.68
		15-18	19.4
	+ve	after 3 hrs.	16.57
0.6	+ve	4-8 min.	17.3
		15-20	24.08
1.2	+ve	4-8	25.48
2.4	+ve	6-12	24.43

DISCUSSION.

It would be found from the experimental results that there is in general an increase in the c.v. values of the silver halides with increasing amounts of the indicator ion. When an acidic indicator is used, the charge of the silver halide has been kept always positive (silver ion in excess $0.1N\text{-KH} = 1.8$ c.c. and $0.1N\text{-AgNO}_3 = 2$ c.c.), while with a basic indicator the charge of the silver halide used is negative (chlorine ion in excess $0.1N\text{-KCl} = 2$ c.c. $0.1N\text{-AgNO}_3 = 1.8$ c.c.). There is to be observed also a time effect.

Considerations of the experimental results with any one single indicator with a particular halide, appear to support the views of the secondary electrical adsorption of the dye. But when we consider the effects observed with a single adsorbent and different indicators, specific nature of adsorption is at once marked. Two types of equilibrium are to be kept in view in explaining the variation of the cataphoretic velocities of silver halides with increasing concentrations of dyestuffs, for when silver nitrate and potassium halides are mixed, the colloidal precipitate that forms takes time to come to equilibrium and to attain its final growth. So the adsorption reaction that takes place between the silver halide and the dyestuff ion is somewhat modified by the velocity of the growth of the particles to its final size, though after some time, the latter effect is practically nil (*cf.* Mukherjee, Chaudhury and Bhabak, *J. Indian Chem. Soc.*, 1936, **13**, 370; Mukherjee, Chaudhury and Sen-Gupta, *ibid.*, 1936, **13**, 421).

My best thanks are due to Prof. J. N. Mukherjee, D.Sc. for facilities given and to Dr. S. G. Chaudhury for suggesting this piece of work.

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Received January 19, 1939.

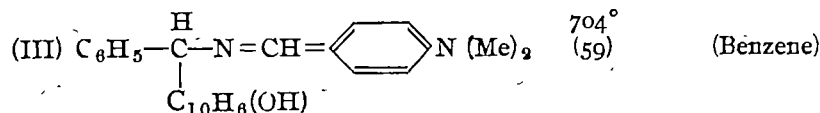
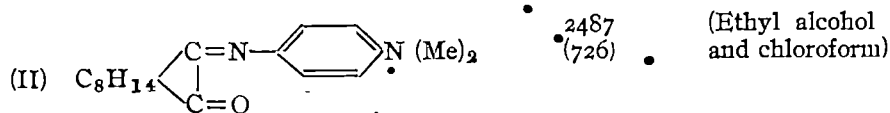
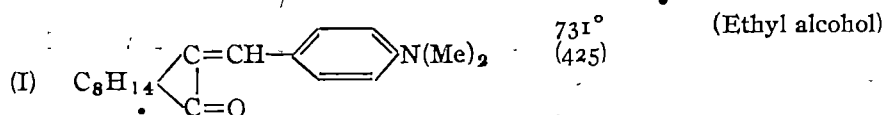
STUDIES ON ROTATORY POWER AND CHEMICAL CONSTITUTION. PART IV.

BY MAHAN SINGH.

This paper describes the condensation products of camphor and *p*-dimethylamino-benzaldehyde and dialkylamino-anilines with (i) camphoric anhydride and (ii) oxymethylene camphor and their rotations. The effect of dimethylamino group in *p*-dimethylaminobenzylidenecamphor is very marked; but the dialkylamino groups in dialkylaminomethylenecamphors have no specific effect. Most of these compounds were examined polarimetrically in the presence of hydrochloric acid and well marked changes in the rotatory power occurred.

The work reported in previous communications (*J. Indian Chem. Soc.*, 1935, **12**, 219, 768; 1936, **13**, 743) has been extended further and three types of compounds have been prepared. Condensation products of (a) camphor and *p*-dimethylaminobenzaldehyde, (b) of camphoric anhydride with dialkylaminoanilines, and (c) of oxymethylenecamphor with the above amines.

Hilditch (*J. Chem. Soc.*, 1909, **95**, 333) has prepared benzylidenecamphor with $[\alpha]_D = 425^\circ$. *p*-Dimethylaminobenzylidenecamphor (I), prepared by treating sodio-camphor with *p*-dimethylaminobenzaldehyde, has $[\alpha]_D = 731^\circ$ (ethyl alcohol). The effect of dimethylamino group is, therefore, very marked. It may be of interest to compare the rotatory power of this compound with those of *p*-dimethylaminophenylisocamphor (II) and *p*-dimethylaminobenzylidene- β -oxynaphthylbenzylamine (III).



The replacement of CH in (I) by the nitrogen atom raised $[\alpha]_D$ from 731° to 2487° . This shows that the auxorotatory effect of the C-C and C-N linkings is not the same.

The rotatory powers of the compounds without the dimethylamino group are shown in parenthesis and it would be seen that in (III) the introduction of this group has raised the rotation twelve times. In (II) and (III) the optical influence is enhanced by the presence of the two groups of the same polarity in the *para*-position.

It is known that well-marked changes in the rotatory power occur when a substituent assumes electric charge by ionisation. Thus, $[\alpha]_D$ of *p*-dimethylaminobenzylidenecamphor in the presence of a few drops of hydrochloric acid fell from 731° to 342.8° and in methyl alcohol with seven drops of methyl iodide it fell from 704° to 621° . It is supposed that the ionisation of the quaternary salt formed is responsible for the fall in rotatory power.

The rotatory power of the *o*-diethylaminocamphoranilic acid is very small and rises considerably on the addition of hydrochloric acid. The values of this acid and those of *o*-dimethyl derivative (*J. Indian Chem. Soc.*, 1936, 13, 744) with and without the addition of hydrochloric acid are given in Table I.

TABLE I.

	MeOH.	EtOH.	Me ₂ CO.
<i>o</i> -Dimethyl	0	0	-9.97°
1 mol. HCl	55.00	51.13	...
<i>o</i> -Diethyl	5.00	2.00	-12.30
1 mol. HCl	38.90	20.00	7.60

The rotatory powers of the *p*-dialkylamino groups together with those of camphoranilic acids are given below.

TABLE II.

	H	N(Me) ₂ .	N(Me)(Et).	N(Et) ₂ .	N(C ₃ H ₇) ₂ .	N(C ₄ H ₉) ₂ .
MeOH	57	69.8	60.65	83.76	44.50	55.40
	—	—	(62.54)	(91.74)	—	(64.4)
EtOH	49.8	62.4	54.60	78.16	51.90	72.70
	—	—	(58.1)	(86.20)	—	—
Me ₂ CO	38.4	54.4	47.78	85.70	43.90	60.00
MeEtCO	—	—	50.05	89.02	—	—

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There is no definite order in which the dialkylamino groups could be arranged with regard to optical rotation. The diethyl compound has the largest value and the dipropyl compound the least. The compounds in some cases were examined polarimetrically in the presence of hydrochloric acid and in every case a slight increase is recorded. These values are given in brackets.

Oxymethylenecamphor readily condenses with primary and secondary amines. The rotations of the condensation products of oxymethylenecamphor with dialkylaminoanilines in various solvents are summarised below.

TABLE III.

Solvent.	N(Me) ₂	N(Me)(Et)	N(Et) ₂	N(C ₃ H ₇) ₂	N(Me) ₂ <i>meta</i>
MeOH	339.67 (244.56)	333.05 (240.30)	303.82 (221.35)	326.30 (291.16)	322.14
EtOH	305.00	330.00	315.93	336.17	340.38
Me ₂ CO	340.00	330.00	375.00	315.00	342.57
MeEtCO	359.90	338.63	366.06	331.99	302.39

These values do not differ very much from the rotatory powers of anilinomethylenecamphor which has $[\alpha]_D = 355.2^\circ$ in ethyl alcohol and 354.3° in acetone (Singh, Bahaduri and Barat, *J. Indian Chem. Soc.*, 1931, 8, 345). The dialkylamino groups in the *p*-position have got no specific effect. The only explanation which can be offered is that the phenyl group is not in close proximity to the asymmetric centre and the dialkylamino groups in the *p*-position are at a considerable distance to exert any influence.

The methyl alcohol solution of the *para* compounds was also examined with 5 drops of (N/2.05) hydrochloric acid and in each case there was a fall in the rotatory power as was expected. The values after the addition of hydrochloric acid are shown in brackets.

The methylenecamphor compounds, when dissolved in benzene and chloroform, become dark coloured and, therefore, could not be examined in these solvents.

EXPERIMENTAL.

p-Dimethylaminobenzylidenecamphor.—Dry camphor (1 mol.) was dissolved in dry ether containing sodium beads (1 mol.) and after cooling

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with ice, dimethylaminobenzaldehyde ($\frac{2}{3}$ mol.) was added when a vigorous reaction set in. The reaction mixture was kept below 5° for 2-3 hours and then refluxed for 20-30 minutes. The reddish brown pasty mass was extracted with ice-cold water and the ethereal layer on evaporation left behind a red viscous oil, which deposited reddish yellow crystals on long standing. These crystals were purified by dissolving in dilute hydrochloric acid and precipitating with a solution of dilute sodium hydroxide. It crystallised from 90% alcohol in bright yellow glistening leaflets, m.p. $141-41.5^{\circ}$. (Found : N, 5.14. $C_{18}H_{26}ON$ requires N, 4.95 per cent). In the above experiment sodium can be replaced by sodium amalgam with advantage; the reaction takes longer time for completion but gives a purer product.

Condensation Products of Camphoric Anhydride with Dialkyl-aminoanilines.—The method of preparation is described in previous papers (J. Chem. Soc., 1925, 127, 1966; 1927, 1994).

p-Methylethylaminocamphoranilic Acid.—Temperature $130-140^{\circ}$, duration of heating 4 hours. It crystallised from dilute alcohol as prisms, m.p. $179-80^{\circ}$. (Found : N, 8.49. $C_{18}H_{28}O_3N_2$ requires N, 8.43 per cent).

p-Diethylaminocamphoranilic Acid.—Condensation temperature was $125-130^{\circ}$. It was purified by repeated dissolution in dilute acetic acid and precipitation with dilute ammonium hydroxide. It was obtained as prisms, m.p. $170.5-171^{\circ}$. (Found : N, 7.90. $C_{20}H_{30}O_3N_2$ requires N, 8.09 per cent).

p-Dipropylaminocamphoranilic Acid.—Temperature, $130-135^{\circ}$, duration of heating 3-4 hours. A micro-crystalline compound with a yellowish tinge was obtained, m.p. 180.5° . (Found : N, 7.37. $C_{22}H_{34}O_3N_2$ requires N, 7.4 per cent).

p-Dibutylaminocamphoranilic Acid.—Temperature, $130-140^{\circ}$, duration of heating 3-4 hours. It was obtained as a crystalline powder with a greyish tinge, m.p. 126° after darkening at $112-113^{\circ}$. (Found : N, 6.87. $C_{24}H_{38}O_3N_2$ requires N, 6.96 per cent).

o-Diethylaminocamphoranilic Acid.—Temperature of condensation was $140-150^{\circ}$. It was obtained as a crystalline powder, shrinking at 147° and melting at $151-53^{\circ}$. (Found : N, 8.25. $C_{20}H_{30}O_3N_2$ requires N, 8.09 per cent).

Condensation of d-Oxymethylenecamphor with p-Aminodialkylanilines.—*d*-Oxymethylenecamphor was prepared by the method of Claisen, Bishop and Sinclair (Annalen, 1894, 281, 331).

p-Dimethylaminoanilinomethylenecamphor.—A mixture of molecular quantities of *d*-oxymethylenecamphor and *p*-aminodimethylaniline, dissolved in the minimum quantities of pure methyl alcohol and dilute acetic

acid respectively, was gently warmed on the water-bath for about 5 minutes. On scratching for a few seconds, the condensation product settled out in a granular form and was crystallised from acetone (charcoal) in prismatic needles, m.p. $173-73.5^{\circ}$ after darkening at 169° . It is readily soluble in acetone and methylethyl ketone, less so in methyl and ethyl alcohol and only sparingly soluble in benzene, and extremely soluble in chloroform and the solution changed in colour from light yellow to light green and ultimately to brown. But these changes were so instantaneous that it was not possible to take the reading in this solvent. It dyes wool yellow. (Found: N, 9.4. $C_{19}H_{26}ON_2$ requires N, 9.39 per cent).

p-Methylethylaminoanilinomethylenecamphor was prepared as in the foregoing experiment by condensing equimolecular proportions of *p*-aminomethylethylaniline and oxymethylenecamphor. It separated from 60-70% alcohol (charcoal) as a crystalline powder, m.p. $132-32.5^{\circ}$ after changing colour at 123° and darkening at $126.5-128^{\circ}$. It is insoluble in water, but it readily dissolves in acetone or methylethyl ketone and is fairly soluble in methyl or ethyl alcohol and very readily in chloroform, the changes in colour being as usual. It dyes wool yellow. (Found: N, 9.12. $C_{20}H_{28}ON_2$ requires N, 8.97 per cent.).

p-Diethylaminoanilinomethylenecamphor was prepared by condensing *p*-dimethylaminoaniline and oxymethylenecamphor as usual. It crystallised from alcohol in prismatic needles, m.p. $134.5-35^{\circ}$. It is insoluble in water, but it dissolves readily in acetone or methyl ethyl ketone and is less soluble in methyl or ethyl alcohol. Its solubility in chloroform is appreciable and the solution undergoes sudden changes in colour and hence failure in taking any reading. The substance in alcoholic solution dyes wool yellow. (Found: N, 8.8. $C_{21}H_{30}ON_2$ requires N, 8.59 per cent.).

p-Dipropylaminoanilinomethylenecamphor.—*p*-Aminodipropylaniline was obtained by preparing the nitroso derivative of dipropylaniline and then reducing the product with stannous chloride and hydrochloric acid. The condensation was effected as in previous cases. The impure product obtained was crystallised twice from 70% alcohol in beautiful yellow crystalline powder, m.p. $136.5-137^{\circ}$ after darkening at $128-29^{\circ}$. The filtrate on dilution gave a very good yield of the substance. It is soluble in the ordinary organic solvents, sparingly in benzene and insoluble in water. Quick changes in chloroform solution are observed. It dyes wool yellow. (Found: N, 8.06. $C_{23}H_{34}ON_2$ requires N, 7.9 per cent.).

Condensation between *d*-oxymethylenecamphor and *p*-aminodiamylaniline under similar conditions gave a viscous mass which could not be crystallised.

m-Dimethylaminoanilinomethylenecamphor.—The mixture of *m*-dimethylaminoaniline and oxymethylenecamphor on prolonged warming and scratching yielded a microcrystalline solid which was crystallised twice from alcohol. It crumbles and becomes slightly yellowish at 158° and finally melts at 159.5–160°. It is soluble in the usual organic solvents but insoluble in water. Unlike *para*-compounds it exhibits no colour changes in chloroform and, therefore, the reading could very easily be taken. It has no dyeing properties. (Found: N, 9.2. $C_{19}H_{28}ON_2$ requires N, 9.39 per cent).

Condensation between *d*-oxymethylenecamphor and *o*-aminomethylaniline gave an oily product which could not be crystallised.

The rotatory powers were determined by dissolving a known weight of the substance in a known volume of the solvent. The following results without any mutarotation, were obtained. The readings within brackets were taken after the addition of hydrochloric acid.

p-Dimethylaminobenzylidenecamphor.

Solvent.	Conc. g./25 c.c.	α_D .	$[\alpha]_D$	Solvent	Conc. g./25 c.c.	α_D .	$[\alpha]_D$
MeOH	0.0926	2.61	704.64	C_6H_6	0.0903	2.22	614.60
		(1.27)	(342.87)				
(on addition of methyl iodide)	0.0978	2.43	621.17	$C_6H_5.CH_3$	0.0852	2.04	598.59
EtOH	0.2000	5.85	731.25	C_6H_5Cl	0.1101	2.95	669.85
Me_2CO	0.1054	2.73	647.53	C_6H_5Br	0.1146	3.19	695.89
MeEtCO	0.0902	2.49	690.13	C_6H_5I	0.1578	3.48	551.33
$CHCl_3$	0.0879	2.42	688.28	$C_6H_5NO_2$	0.1165	3.24	695.28

p-Methylethylamino-camphoranilic acid.

p-Diethylamino-camphoranilic acid.

				MeOH	0.2507	0.84	83.76
						(0.92)	(91.74)
MeOH	0.2638	0.64	60.65	EtOH	0.2175	0.68	78.16
		(0.66)	(62.54)			(0.75)	(86.21)
EtOH	0.2880	0.63	54.60	MeEtCO	0.2050	0.73	89.02
		(0.67)	(58.16)	Me_2CO	0.2100	0.72	85.71
Me_2CO	0.2093	0.40	47.78	$CHCl_3$	0.2000	0.65	81.25
MeEtCO	0.2048	0.41	50.05				

p-Dipropylaminocamphoranilic acid.

Solvent	Conc. g / 25 c.c.	α_D	$[\alpha]_D$
MeOH	0.1293	0.23	44.50
EtOH	0.1348	0.28	51.90
Me ₂ CO	0.1308	0.23	43.90

*p-Dibutylaminocamphoanilic acid.**o-Dimethylaminocamphoranilic acid.*

Solvent.	Conc. g/25 c.c.	α_D	$[\alpha]_D$	Conc. g / 25 c.c.	α_D	$[\alpha]_D$
MeOH	0.1670	0.37	55.40	0.2000	0	0
	"	(0.43)	(64.40)	0.3000	0	0
EtOH	0.1718	0.50	72.70	0.2006	-0.08	-9.97
Me ₂ CO	0.1705	0.41	60.00			
(Acid equiv. added in MeOH)				0.2000	0.44	55.00
(Acid equiv. added in EtOH)				0.2000	0.41	51.13

*o-Diethylaminocamphoranilic acid.**p-Dimethylaminoanilinomethylene-camphor.*

MeOH	0.2506	0.05	5.00	0.0552	0.75	339.67
		(0.39)	(38.90)		(0.54)	(244.56)
EtOH	0.2500	0.02	2.00	0.0500	0.61	305.00
		(0.20)	(20.00)			
Me ₂ CO	0.2628	-0.13	-12.30	0.0500	0.68	340.00
		(0.08)	(7.60)			
MeEtCO	0.2441	-0.12	-12.29	0.0550	0.79	359.90

*p-Methylethylaminoanilinomethylene-camphor.**p-Diethylaminoanilinomethylene-camphor.*

MeOH	0.0593	0.79	333.5	0.0576	0.70	303.82
		(0.57)	(240.30)		(0.51)	(221.35)
EtOH	0.0500	0.66	330.00	0.0536	0.69	315.93
Me ₂ CO	0.0500	0.66	330.00	0.0500	0.71	375.00
MeEtCO	0.0550	0.745	338.63	0.0601	0.88	366.06

p-Dipropylaminoanilinomethylene-
camphor. *m*-Dimethylaminoanilinomethylene-
camphor.

Solvent	Conc. g./25 c.c.	n_D	$[\alpha]_D$	Conc. g./25 c.c.	n_D	$[\alpha]_D$
MeOH	0.0498	0.65	326.30	0.0551	0.71	322.14
		(0.58)	(291.16)	0.0522	0.71	340.38
EtOH	0.0528	0.71	336.17	0.0613	0.84	342.57
Me ₂ CO	0.0500	0.63	315.00		(0.92)	(375.20)
MeEtCO	0.0497	0.76	331.99	0.0677	0.83	302.31
					(0.91)	(336.06)
CHCl ₃				0.0754	0.85	281.83

The readings were taken in dark room, the temperature varying from 19-22°.

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Received October 31, 1938

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS. PART IV. OXIDATION WITH POTASSIUM CHLORATE.

BY BALWANT SINGH AND SOHAN SINGH.

Potassium iodide, ferrous ammonium sulphate, thallous chloride, arsenious oxide and potassium antimonyl tartrate were titrated potentiometrically against standard potassium chlorate in presence of a large excess of hydrochloric acid. With the addition of potassium chlorate, E.M.F. was found to rise steadily except in arsenious oxide where it did not change but near the equivalence point. At the equivalence point there was a sharp jump in potential followed by a steady rise in each case.

Potassium bromate and potassium iodate, especially the latter, have been extensively used as reagents in quantitative analysis. Practically no work has been done with potassium chlorate as a volumetric reagent in oxidation-reduction reactions.

An aqueous solution of potassium chlorate is fairly stable and reacts slowly with dilute hydrochloric acid with the evolution of chlorine. Raising the temperature, increases the rate of evolution of the gas; and the reaction is accelerated with platinum as catalytic agent.

In the present investigation potassium iodide, ferrous, thallous, arsenious and antimonious compounds have been determined potentiometrically by titrating them against standard potassium chlorate in presence of a large excess of hydrochloric acid.

EXPERIMENTAL.

The oxidation-reduction electrode, which consisted of a bright platinum foil, immersed in the solution to be titrated, was coupled with a saturated calomel electrode. The cell was placed in a water-bath, temperature of which was kept at 25°.

A known weight of each salt was weighed into a titration vessel and the required amount of concentrated hydrochloric acid added to keep its concentration above 5 N. Standard potassium chlorate was added from a burette, the mixture stirred by a mechanical stirrer and the progress of oxidation followed with the potentiometer.

Potassium iodide and ferrous ammonium sulphate were titrated in an atmosphere of carbon dioxide to prevent oxidation by the atmospheric air. Iodine monochloride has been used as a catalyst in the case of

thallous chloride and arsenious oxide. In the case of potassium iodide, the temperature of the bath was 10° . In the case of potassium antimonyl tartrate, the temperature of the bath was kept at 45° , as the reaction was very slow at 25° .

A series of potentiometric titrations were performed with different amounts of each substance. One titration, as typical of that set, is recorded in the following table.

TABLE I.

0.6086 G. of potassium iodide dissolved in 10 c.c. of water, and 50 c.c. of conc. hydrochloric acid titrated against $M/20$ -potassium chlorate.

KClO ₃ .	E.M.F.	E/C.	KClO ₃ .	E.M.F.	E/C.
7.000 c.c.	0.250 volt		24.200	0.634 volt	
10.000	0.302	17 ml. volt/c c.	24.300	0.646	120 ml. volt/c
11.000	0.321	19	24.350	0.654	160
12.000	0.404	83	24.375	0.662	320
13.000	0.506	102	24.400	0.678	640
14.000	0.526	20	24.425	0.814	5440 (maximum)
16.000	0.546	10	24.450	0.830	640
20.000	0.570	6	24.500	0.840	200
22.000	0.585	7	24.600	0.856	160
23.000	0.598	13	24.800	0.876	100
23.600	0.610	20	25.300	0.890	28
24.000	0.620	25	26.300	0.898	8
		70			

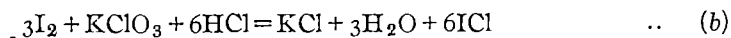
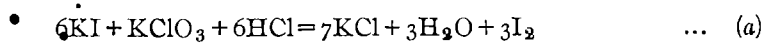
The titrations, one for each substance, are represented by the curves given in page 29.

D I S C U S S I O N.

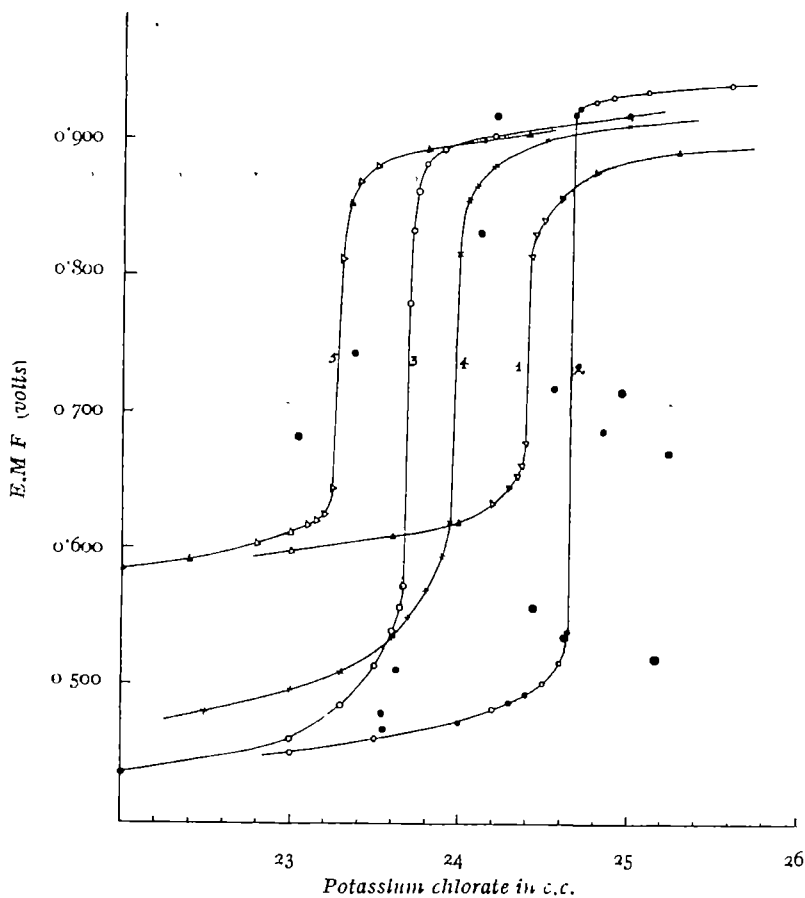
In these titrations, with the addition of the standard potassium chlorate solution, the E.M.F. rose steadily except in arsenious oxide, where it did not change but near the equivalence point. At the equivalence point there was a sharp jump in potential, followed by a steady rise in each case.

In the case of potassium iodide, another break in the E.M.F. was observed when nearly half of the volume of the titrant required for the equivalence point was added to the reaction mixture.

The reaction between potassium iodide and potassium chlorate in the presence of a large excess of hydrochloric acid takes place in two stages as follows



The completion of each of these stages is marked by a break in the E.M.F. The first break takes place when all the iodide is converted into iodine and the second break corresponding to the equivalence point, is observed when all the iodine, liberated during the first stage, is changed to iodine monochloride.



Curves 1—5 refer respectively to potassium iodide, ferrous am. sulphate, thallous chloride, arsenious oxide, pot. antimonyl tartrate.

From the volume of potassium chlorate required in each titration, corresponding to the equivalence point, the amount of the compound was calculated. In the following table the values obtained are compared with the amount of the substance taken.

TABLE II.

Potassium iodide. (taken). (found).	Ferrous Am. sulphate (taken). (found).	Thallous chloride. (taken). (found)	Arsenious oxide (taken). (found).	antim. tartrate (taken). (found)
0.6086g. 0.6080 g.	1.7394 g. 1.7388 g.	1.0780 1.0778	0.4452 0.4444	1.5102 1.5096
0.4950 0.4945	0.9675 0.9670	0.7018 0.7015	0.2445 0.2438	0.9123 0.9121
0.3595 0.3593	0.5780 0.5775	0.4854 0.4848	0.2226 0.2221	0.5672 0.5671
0.2002 0.1998	0.4571 0.4567	0.2850 0.2844	0.1335 0.1333	0.3196 0.3196
0.1660 0.1656		0.1798 0.1794	0.0890 0.0888	0.2442 0.2441

These results show that potassium iodide, ferrous ammonium sulphate, thallous chloride, arsenious oxide and potassium antimonyl tartrate can be potentiometrically determined by using potassium chlorate as the titrating agent.

The authors thank the Khalsa College authorities for a research grant.

DEPARTMENT OF CHEMISTRY,
KHALSA COLLEGE,
AMRITSAR.

Received December 5, 1938.

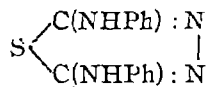
THE PHENYLTHIOCARBAMIDES. A CONTRIBUTION TO
THE STUDY OF THE TRIAD -N·C·S-. PART VIII.
THE CHEMISTRY OF HECTOR'S BASE AND
ATTEMPTS TOWARDS ITS SYNTHESIS.

BY KUNJ BREHARI LAL AND HANS KRALL.

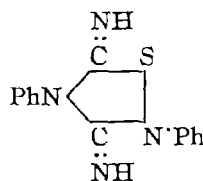
Hector's base, $C_{14}H_{12}N_4S$ (I) is formed very readily on oxidation of phenylthiocarbamide (II) in presence of a little acid and may be expected to throw light on the constitution of (II). Contrary to Fromm's supposition (*Ber.*, 1909, **42**, 3804) (I) appears to be a direct oxidation product of (II). This view is suggested by the negative results obtained by oxidising together, (i) (II) and phenylcyanamide, (ii) monophenylguanidine and phenylmustard oil and, (iii) diphenylguanidine and thiocyanic acid respectively. The above theory has been further supported by the reaction between nitrous acid and (II) studied in presence of relatively concentrated acid.

Hector (*Ber.*, 1889, **22**, 1176) obtained a base ($C_{14}H_{12}N_4S$) on oxidising phenylthiocarbamide with hydrogen peroxide in presence of a little acid. Subsequent workers obtained it with other oxidising agents, *e.g.*, bromine in alcoholic solution (Hugershoff, *Ber.*, 1901, **34**, 3130), nitrous acid (Haager and Dohrt, *Monatsh.*, 1906, **27**, 267), *p*-toluenesulphuryl chloride (Fromm and Heyder, *Ber.*, 1909, **42**, 3804), and catalytic oxidation with air (Fruendlich and Bjerke, *Z. physikal. Chem.*, 1916, **9**, 1). The chemistry of the base does not seem to be well known. It is, however, astonishing to find that copper sulphate, a reagent less known for its oxidising properties, yields the base from phenylthiocarbamide (*cf.* Lal and Krall, *J. Indian Chem. Soc.*, 1937, **8**, 477), whilst silver nitrate fails to do so.

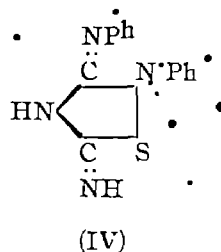
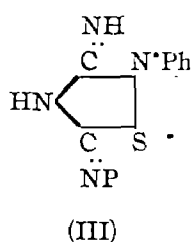
Hector originally represented the base by the structure (I) and named it dianilino-*o*-diazothiol or diphenyldiamido-*o*-diazothiol according to Widman's nomenclature (*J. pr. Chem.*, 1892, *ii*, **48**, 200), but later considered it to possess structure (II) of an azosulphine type probably on the suggestion of Hofmann and Gabriel (*Ber.*, 1892, **25**, 1578). Dost (*Ber.*, 1906, **39**, 863) on hydrolysing the base with fuming hydrochloric acid found that only one :NH group could be split off, the hydrolytic product being a non-basic keto compound.



(I)

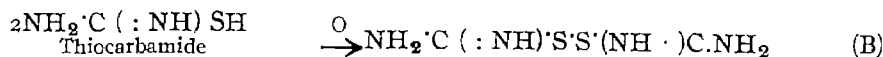
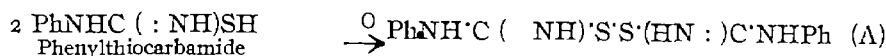


(II)



He, therefore, favoured structure (III) which contains only one imino group, though the possibility of structure (IV) is not thus precluded.

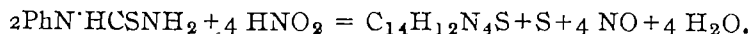
With regard to the mechanism of its formation, Fromm (*loc. cit.*) like other workers supposed that the first product of oxidation of phenylthiocarbamide was actually a disulphide (A) in analogy with the case of thiocarbamide (*cf.* Werner, *J. Chem. Soc.*, 1912, 101, 2180), thus



The compound (A) was then considered to decompose into sulphur, phenylthiocarbamide, and phenylcyanamide, from which the diazothiol was ultimately built up. From this theory it occurred to the present authors that it should be possible to get Hector's base from suitable residues of compounds (*vide infra*) if the base represents a stable configuration built up of simpler molecules. The present communication records the results of such attempts and suggests that the base is a direct oxidation product of phenylthiocarbamide. This is further supported by the action of nitrous acid on phenylthiocarbamide (Mehta and Krall, *J. Indian Chem. Soc.*, 1935, 12, 635)

According to Fromm (*loc. cit.*) phenylthiocarbamide and phenylcyanamide, when oxidised together, should yield Hector's base. When, however, these two compounds in alcoholic solution are oxidised in presence of a little acid with hydrogen peroxide (*vide experimental*), sulphur separates and the yield of the base is not greater than that obtainable without the cyanamide. Monophenylguanidine and phenylmustard oil give a non-basic compound (m.p. 198°) which does not produce Hector's base on oxidation. The possibility of the alternative structure (IV) was tested by reacting diphenylguanidine with thiocyanic acid, the product being diphenylguanidine thiocyanate, which on oxidation did not give Hector's base. The amounts of the separated sulphur and of Hector's base in the action of sodium nitrite

on phenylthiocarbamide (*cf.* Mehata and Krall, *loc. cit.*) correspond to the direct oxidation of about half of the thiocarbamide according to the following equation



From this fact it appears that Hector's base is not a secondary product as supposed by Fromm.

EXPERIMENTAL.

Oxidation of Phenylcyanamide and Phenylthiocarbamide.—Moist phenylcyanamide (2.5 g.) was added to a solution of phenylthiocarbamide (2.5 g.) in alcohol (70 c.c.). The solution was acidified with hydrochloric acid (1 c.c.). A 3% solution of hydrogen peroxide (10-12 g.) was gradually added when sulphur separated. The mixture was diluted to about 150 c.c. with water and filtered from separated sulphur (0.2 g.). The filtrate, which was slightly cloudy (phenylcyanamide), was basified with alkali which precipitated Hector's base (1.8 g.; theoretical yield without using the cyanamide being 2.1 g.). The phenylcyanamide appeared to remain unchanged. The result was identical when dilute alcoholic solutions were employed.

Oxidation of Monophenylguanidine and Phenylmustard Oil.—The guanidine was prepared (a) from cyanamide and aniline hydrochloride (Kampf, *Ber.*, 1904, 37, 1681) and also (b) by a method based on that of Werner (*J. Chem. Soc.*, 1922, 1790). The melt was extracted with water and the aqueous extract basified with alkali. In each case monophenylguanidine was isolated from basified solution as carbonate (m.p. 138°) by precipitation with a solution of saturated potassium carbonate, identified through its benzoyl derivative (m.p. 186°). An alcoholic suspension of phenylguanidine carbonate (1.66 g.) was neutralised with acetic acid and mixed with an alcoholic solution of phenylmustard oil (1.35 g.). After warming for 14 minutes the mixture was treated with 3% hydrogen peroxide (6.8 g.). When placed overnight it gave needle-shaped crystals of a non-basic compound m.p. 198°, containing S and N, which was not Hector's base. The compound gave an acetyl derivative (m. p. 235°) and could also be obtained from the above constituents without the addition of hydrogen peroxide.

Oxidation of Diphenylguanidine and Thiocyanic Acid.—Equivalent quantities of diphenylguanidine and thiocyanic acid in ethereal solution were mixed when diphenylguanidine thiocyanate (m. p. 115°) was obtained. This did not give Hector's base on oxidation with hydrogen peroxide and remained unchanged on heating.

Action of Nitrous Acid on Phenylthiocarbamide dissolved in concentrated Hydrochloric Acid.—Phenylthiocarbamide (3.04 g.) was dissolved in concentrated hydrochloric acid (16 c.c.) in a separating funnel. The solution was treated slowly with aqueous sodium nitrite (20 c.c. *M.*), and the contents of the separating funnel were cooled by running water (18–19°). The reaction began with brisk evolution of gas and the solution smelt of isocyanide and of phenylmustard oil. The small amount of the oily and resinous mass was removed by extraction with 5 c.c. of chloroform thrice. The aqueous layer remained clear for 2 hours and deposited sulphur (0.2 g; theoretical 0.32 g.) on dilution and slight warming. The filtrate from it contained sulphuric acid (3.4%), Hector's base (1.05 g, identified through its nitroso derivative and the acetyl derivative) and unchanged thiocarbamide.

In conclusion it might be mentioned that Barnett (*J. Chem. Soc.*, 1910, 97, 63) only obtained an oil on oxidising phenylthiocarbamide in the absence of an acid and not Hector's base. With thiocarbamide under this condition, the product according to him was a derivative of sulphinic acid which has been further investigated by Boesken (*Proc. K. Akad. Wetensch. Amsterdam*, 1936, 39, 717; *Rec. trav. chim.*, 1936, 55, 1040, 1044). Oxidation reactions of the thiocarbamides are proposed to be further investigated.

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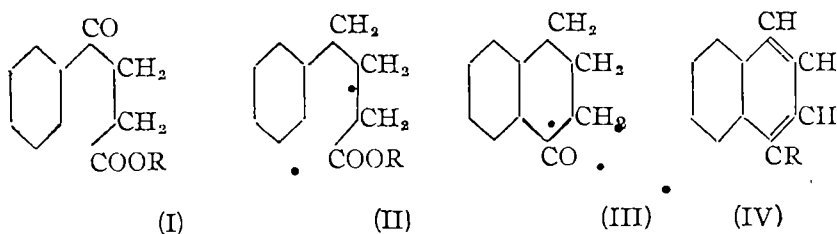
Received December 23, 1938.

STUDIES IN γ -KETONIC ACIDS. PART I.

By P. C. MITTER AND SHYAMAKANTA DE.

Bürker (*Ann. chim. phys.*, 1882, **26**, v, 435) first effected the condensation between benzene and succinic anhydride in presence of anhydrous aluminium chloride and obtained β -benzoylpropionic acid. This opened up a new route for synthesising β -aroylpropionic acids and substituted β -aroylpropionic acids by using the appropriate components.

Clemmensen reduction of the β -aroylpropionic acids (I, R=H) gives rise to γ -arylbutyric acids (II, R=H) which can be cyclised to the corresponding 1-ketotetrahydronaphthalene (III). Further Clemmensen reduction of the keto compound leads to the formation of a tetrahydronaphthalene which can be dehydrogenated with selenium to the corresponding naphthalene derivative.* Alternatively, a new alkyl group can be introduced by the action of the Grignard reagent on the ester of β -aroylpropionic acid, (I, R=alkyl) followed by dehydration, dehydrogenation and ring closing when 1-alkyl-4-ketotetrahydronaphthalene derivative is obtained. A



repetition of the same series of reactions would lead to 1:4-dialkyltetrahydronaphthalene derivative.

We have studied the condensation of phenol and various phenolic ethers with succinic anhydride with the object of synthesising various naphthalene derivatives and also with a view to fill up *lacunae* in the works of other authors. An attempt has also been made to prepare acenaphthene derivatives by condensing 7-methoxy-1-ketotetrahydronaphthalene with bromoacetic ester in the presence of zinc, and subsequent ring-closing, but

* On the other hand, by the action of the Grignard reagent on the keto-compound followed by dehydrogenation and selenium treatment an α -alkyl naphthalene (IV) may be obtained

the condensation by Reformatsky's reaction resulted in such a poor yield that further progress was rendered impossible.

Rosenmund and Schapiro (*Arch. Pharm.*, 1934, 272, 313) have found that anisole condenses with succinic anhydride in presence of anhydrous aluminium chloride in nitrobenzene medium to give γ -*p*-methoxyphenyl- γ -ketobutyric acid. We have found that the reaction proceeds smoothly in acetylene tetrachloride medium at the ordinary temperature (*i.e.* 25°) with almost theoretical yield. The keto-acid gives a semicarbazone (m.p. 185-86°).

By the reduction of this keto-acid by Clemmensen's original method (*Ber.*, 1913, 46, 1837) Haworth and Sheldrick (*J. Chem. Soc.*, 1934, 1951) obtained γ -*p*-methoxyphenylbutyric acid (m.p. 63-64°). They converted the acid into the chloride and effected the ring-closing with the help of anhydrous aluminium chloride. The ring-closure proceeds smoothly with almost theoretical yield with phosphorus pentoxide in benzene medium and gives 7-methoxy-1-keto-1:2:3:4-tetrahydronaphthalene (m.p. 62°, semicarbazone, m.p. 221°). Further reduction of the ring ketone by Clemmensen's method gives 1:2:3:4-tetrahydronaphthalene-7-methyl ether (b. p. 103°-105°/5.5 mm.) identical with the compound obtained by Schroeter and others (*Annalen*, 1922, 426, 83) by methylating tetrahydro- β -naphthol with dimethyl sulphate and alkali.

1-Keto-7-methoxy-1:2:3:4-tetrahydronaphthalene gives on condensation with methyl magnesium iodide the completely dehydrated product, 1-methyl-7-methoxy-3:4-dihydronaphthalene (b. p. 124°/5.5 mm.), which on dehydrogenation with selenium (Diels, Gadke and Kording *Annalen*, 1927, 469, 1), gives 1-methyl-7-methoxynaphthalene (m. p. 46°). Haworth and Sheldrick (*loc. cit.*) did not isolate the intermediate product. They submitted the product obtained by treatment with the Grignard reagent direct to selenium dehydrogenation and obtained 1-methyl-7-methoxynaphthalene (m. p. 47°-48°; picrate, m.p. 117°). Again 1-methyl-7-methoxy-3:4-dihydronaphthalene on catalytic hydrogenation readily gives 1-methyl-7-methoxy-1:2:3:4-tetrahydronaphthalene (b. p. 116°/8 mm.).

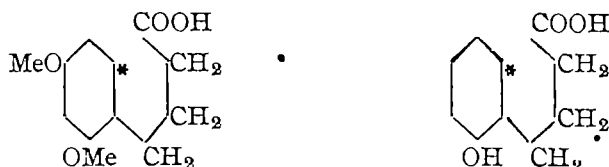
Pyrogallol trimethyl ether condenses with succinic anhydride in presence of anhydrous aluminium chloride in acetylene tetrachloride to give γ -2-hydroxy-3:4-dimethoxyphenyl- γ -ketobutyric acid (m. p. 152°), one methyl group being knocked off during the reaction. The keto-acid on reduction by Clemmensen's method gives γ -2-hydroxy-3:4-dimethoxyphenylbutyric acid (m. p. 103°), dehydration of which with 85% sulphuric acid gives 1-keto-1:2:3:4-tetrahydro-5-hydroxy-6:7-dimethoxynaphthalene (m. p. 155°; semicarbazone, m.p. 226°). Further reduction of this ring-ketone by

Clemmensen's method gives 1:2:3:4-tetrahydro-5-hydroxy-6:7-dimethoxynaphthalene.

Perkin and Ormerod (*J. Chem. Soc.*, 1902, 81, 234) condensed resorcinol dimethyl ether with the ester of half chloride of succinic acid in a mixture of nitrobenzene and carbon disulphide in presence of anhydrous aluminium chloride and obtained in very poor yield 2:4-dimethoxybenzoylpropionic acid after hydrolysing the resulting crude ester. Perkin and Robinson (*J. Chem. Soc.*, 1908, 98, 506, *cf.* Dalal and Nargund, *J. Indian Chem. Soc.* 1937, 14, 408) obtained the same acid with apparently better yield by effecting condensation in carbon disulphide medium or without any solvent. But they did not mention the formation of any other product in this reaction. We have found that resorcinol dimethyl ether condenses readily with succinic anhydride in presence of anhydrous aluminium chloride in acetylene tetrachloride medium at 50-60° and the product after crystallisation from water does not melt at a definite temperature and it gives ferric chloride colouration. The distilled product is dissolved in ether, and washed with ice-cold dilute caustic soda solution. On acidification with hydrochloric acid in the cold γ -2-hydroxy-4-methoxyphenyl- γ -ketobutyric acid is obtained identical with the acid prepared by Perkin and Robinson (*loc. cit.*) under more drastic conditions. The ester obtained after distilling off the ether is the ester of γ -2:4-dimethoxyphenyl- γ -ketobutyric acid (m.p. 66°); the hydrolysis of which gives γ -2:4-dimethoxyphenyl- γ -ketobutyric acid (m.p. 147°; semicarbazone, m.p. 166°). Reduction of this acid by Clemmensen's method gives γ -2:4-dimethoxyphenylbutyric acid (m.p. 48°). Attempts have been made to cyclise this acid with dehydrating agents like phosphorus pentoxide, concentrated sulphuric acid, fuming stannic chloride, fused zinc chloride and by treating the chloride of this acid with anhydrous aluminium chloride but without success.

Phenol condenses with succinic anhydride in presence of anhydrous aluminium chloride in acetylene tetrachloride medium at 130°-140° and gives rise to γ -2-hydroxyphenyl- γ -ketobutyric acid (m.p. 145°), identical with the acid previously obtained by Rosenmund and Schapiro (*loc. cit.*) from 2-oxy- β -chloropropionylbenzol of Meyer and Zutphen (*Ber.*, 1924, 57, 200) over the nitrile. Reduction of this keto-acid by Clemmensen's method gives rise to γ -o-hydroxyphenylbutyric acid (m.p. 67°) with almost theoretical yield, identical with the compound obtained by Schroeter (D.R.P., 562,827, Dec. 21, 1928) from 5-keto-tetrahydronaphthalene. This acid could not be cyclised by dehydration with concentrated sulphuric acid, phosphorus pentoxide, fuming stannic chloride or by the acid chloride method.

We have observed that γ -2,4-dimethoxyphenylbutyric acid and γ -*o*-hydroxyphenyl-butylric acid could not be cyclised by various dehydrating agents. This is most probably due to the presence of *ortho* or *para* directing group *meta* to the position marked * in which cyclisation must take place as will be evident from the formulæ.



These are not the only instances where similar orienting influence has inhibited cyclisation. Numerous other examples might be cited from the works of other authors. Thus Walsh and Weizmann (*J. Chem. Soc.*, 1910, 97, 685), Graves and Adams (*J. Amer. Chem. Soc.*, 1923, 45, 2439), Gardner and Adams (*ibid.*, 1923, 45, 2455), and Jacobson and Adams (*ibid.*, 1924, 46, 1312) observed a similar difficulty in the formation of anthraquinones from those benzoylbenzoic acids which contain an *ortho* or *para* directing group *meta* to the position in which condensation must take place. This difficulty was particularly pronounced in the case of phenol derivatives probably on account of the ease with which sulphonation took place. Attempts made by Feiser and Bradsher (*J. Amer. Chem. Soc.*, 1936, 58, 1738) to cyclise the butyric acid derivatives of diphenyl namely, γ -(4-methoxy-3-phenyl)-butyric acid, with sulphuric acid through the acid chloride were unsuccessful and they ascribed their failure to the above causes.

EXPERIMENTAL.

γ -*p*-Methoxyphenyl- γ -ketobutyric Acid—Powdered anhydrous aluminium chloride (120 g.) was slowly added to a mixture of anisole (47.5 g.), succinic anhydride (40 g.) and acetylene tetrachloride (160 c.c.) kept cool in ice-water. The temperature was not allowed to rise above 40°. The mixture was left overnight and then decomposed by the addition of ice and dilute (1:1) hydrochloric acid. The excess of anisole and acetylene tetrachloride were removed by distillation in steam. The residue in the flask solidified on cooling. It was filtered, digested with sodium carbonate solution and the filtrate was acidified with concentrated hydrochloric acid in the cold, when the keto-acid was precipitated. It was crystallised from water, m.p. 146°, yield 67 g. (Haworth and Sheldrick gives m.p. 147-48°).

(Found: C, 63.33; H, 5.72. Calc. for $C_{11}H_{12}O_4$: C, 63.46; H, 5.76 per cent). The keto-acid gave a semicarbazone which was crystallised from alcohol, m. p. 185-86°. (Found: N, 16.07. $C_{12}H_{15}O_4N_3$ requires N, 15.88 per cent).

γ -p-Methoxyphenylbutyric Acid.—The keto-acid (40 g.) was reduced with zinc amalgam prepared, from zinc filings (120 g.), and hydrochloric acid by heating for 8 hours. The product which solidified when kept overnight was dissolved in sodium carbonate and the solution precipitated with hydrochloric acid. The acid crystallised from petroleum ether (b.p. 60-80°) in beautiful crystals, m. p. 61°, yield theoretical. (Haworth and Sheldrick gives m. p. 63-64°) (Found: C, 68.41, H, 7.02. Calc. for $C_{11}H_{14}O_3$: C, 68.04; H, 7.21 per cent).

1-Keto-1:2:3:4-tetrahydro-7-methoxynaphthalene.—The above acid (20 g.) dissolved in anhydrous benzene (100 c.c.), was heated on the boiling water-bath with phosphorus pentoxide (60 g.). After removing benzene, the residue was treated with powdered ice and distilled in steam, when the ketone was obtained as an oil in the distillate. It was extracted with ether and on removing ether it solidified when kept in a vacuum desiccator. It crystallised from petroleum ether (b. p. 60-80°) in square plates, m. p. 62°, yield 15 g. (Haworth and Sheldrick gives m. p. 66-67°). (Found: C, 75.32; H, 6.58. Calc. for $C_{11}H_{12}O_2$: C, 75.00; H, 6.81 per cent). The semicarbazone crystallised from alcohol, m. p. 221°. (Found: N, 18.32. $C_{12}H_{15}O_2N_3$ requires N, 18.02 per cent).

1:2:3:4-Tetrahydro-7-methoxynaphthalene.—The above ketone (8 g.) was heated with amalgamated zinc (35 g.) and hydrochloric acid for 24 hours. The product was extracted with ether, the extract washed with water, dried and distilled at 103.5°/5.5 mm.; yield 5 g. (Found: C, 81.66; H, 8.61. $C_{11}H_{14}O$ requires C, 81.48; H, 8.64 per cent).

Condensation of Methyl Magnesium Iodide with 1-Keto-7-methoxy-1:2:3:4-tetrahydronaphthalene.—The ketone (27 g.), dissolved in anhydrous ether (100 c.c.), was added to the Grignard reagent prepared from 5 g. of magnesium and 22 c.c. of methyl iodide in equal volume of anhydrous ether. The mixture was left overnight and the magnesium compound decomposed with ice cold 10% dilute sulphuric acid and then extracted with ether. The ethereal extract, washed with sodium bisulphite and water was distilled at 124°/5.5 mm., yield 21.5 g. The distilled product turned pale brown within 15 minutes and was found by analysis to be completely dehydrated. It decolourised potassium permanganate solution almost instantaneously. (Found: C, 82.47; H, 8.24. $C_{12}H_{14}O$ requires C, 82.75; H, 8.04 per cent).

1-Methyl-7-methoxynaphthalene.—The above product was dehydrogenated with selenium in the usual manner by heating for 30 hours at 310–330°. The product was extracted with ether, washed with 5 % caustic soda solution, then with water and finally the ethereal solution was dried and distilled at 134°/7mm. as a colourless liquid which solidified when kept in a vacuum desiccator, m.p. 46°, yield 4.5 g. (Haworth and Sheldrick gives m.p. 47–48°). (Found: C, 83.69, H, 7.09. Calc. for $C_{12}H_{12}O$: C, 83.72; H, 6.97 per cent). The picrate crystallised from absolute alcohol as orange needles, m.p. 117°. (Found: N, 10.42. $C_{18}H_{15}O_8N_3$ requires N, 10.47 per cent).

Catalytic Hydrogenation and Formation of 1-Methyl-7-methoxy-1:2:3:4-tetrahydronaphthalene.—Freshly distilled 1-methyl-7-methoxy-3:4-dihydronaphthalene (9.5 g.) in alcohol (30 c.c.) was hydrogenated in presence of 0.2 g. platinum oxide. The calculated quantity of hydrogen was practically absorbed within an hour. The product was then transferred into the conical flask and the portion adhering to the flask was extracted with ether and then dried over anhydrous sodium sulphate. Ether and alcohol were removed and the reduced product was distilled at 116°/8 mm., yield 8 g. (Found: C, 81.54; H, 9.07. $C_{12}H_{16}O$ requires C, 81.81; H, 9.09 per cent).

γ -2-Hydroxy-3:4-dimethoxyphenyl- γ -ketobutyric acid was obtained from pyrogallol trimethyl ether (52 g.), succinic anhydride (30 g.) and acetylene tetrachloride (120 c.c.) using anhydrous aluminium chloride (90 g.), yield 51 g. It crystallised from water as colourless needles, m.p. 152°. It gives violet colouration with ferric chloride. (Found: C, 56.55; H, 5.34. $C_{12}H_{14}O_6$ requires C, 56.69; H, 5.51 per cent).

γ -2-Hydroxy-3:4-dimethoxyphenylbutyric acid was prepared from the above keto-acid (30 g.) by heating with amalgamated zinc (90 g.) and hydrochloric acid for 8 hours. The product was extracted with ether, the extract washed with water, dried and the ether removed. The residue solidified in a vacuum desiccator, yield almost quantitative. It was crystallised from petroleum ether (b.p. 60–80°), m.p. 103°. (Found: C, 60.41; H, 6.76. $C_{12}H_{16}O_5$ requires C, 60.00; H, 6.66 per cent).

1-Keto-1:2:3:4-tetrahydro-5-hydroxy-6:7-dimethoxynaphthalene.—The above acid (6 g.) and sulphuric acid (85%, 25 c.c.) was heated on the water-bath for about 1 hour. The mixture was then poured into ice and the product was extracted with ether, the extract washed with sodium bicarbonate solution and with water. The ethereal solution was dried and the ether removed. The ring ketone crystallised from ether in colourless fine needles, m.p. 155°, yield 4 g. (Found: C, 65.04; H, 6.37. $C_{13}H_{14}O_4$

requires C, 64.86; H, 6.30 per cent). The *semicarbazone* crystallised from alcohol in brown crystals, m.p. 226°. (Found: N, 15.51. $C_{13}H_{17}O_4N_3$ requires N, 15.05 per cent).

1:2:3:4-*Tetrahydro-5-hydroxy-6,7-dimethoxynaphthalene*.—The preceding ring ketone (6 g.) was heated for 24 hours with amalgamated zinc (30 g.) and concentrated hydrochloric acid. The product was extracted with ether, and the ethereal solution dried, ether removed and the residue distilled at 148–154°/5.5 mm, yield 3 g. (Found: C, 69.51; H, 7.50. $C_{12}H_{16}O_3$ requires C, 69.23; H, 7.69 per cent).

Condensation of Succinic Anhydride with Resorcinol Dimethyl Ether.—Powdered anhydrous aluminium chloride (120 g.) was gradually added to a mixture of succinic anhydride (40 g.), resorcinol dimethyl ether (60 g.) and acetylene tetrachloride (160 c.c.) with constant shaking. The mixture was then heated at 50–60° for 3 hours, kept overnight and then treated with powdered ice and dilute hydrochloric acid (1:1). Excess of resorcinol dimethyl ether and acetylene tetrachloride were removed by steam distillation. The solid residue was dissolved in sodium carbonate solution and the mixture of keto-acids precipitated by acidification with hydrochloric acid, yield 85 g.

γ -2-Hydroxy-4-methoxyphenyl- γ -ketobutyric Acid.—The crude mixture (40 g.) of keto-acids was refluxed for about 8 hours on the sand-bath with absolute alcohol (100 c.c.) and concentrated sulphuric acid (10 c.c.). Excess of alcohol was removed by distillation and the residue extracted with ether, the extract washed with sodium bicarbonate solution and then with 10% dilute ice-cold caustic soda solution. The caustic soda solution on acidification with cold concentrated hydrochloric acid gave not the ester but the keto-acid. It was crystallised from water, m.p. 154°, yield 5 g. It gives violet colouration with ferric chloride. (Found: C, 59.12; H, 5.52. $C_{11}H_{12}O_5$ requires C, 58.92; H, 5.35 per cent). The *semicarbazone* was crystallised from glacial acetic acid, m.p. 198°. (Found: N, 14.69. $C_{12}H_{16}O_5N_3$ requires N, 14.94 per cent).

Ethyl γ -2:4-Dimethoxyphenyl- γ -ketobutyrate.—The above ethereal solution was dried, ether removed and the residue was distilled at 198–200°/5 mm., m.p. 66°, yield 32 g., (Found: C, 63.49; H, 6.93. $C_{14}H_{18}O_5$ requires C, 63.15; H, 6.76 per cent).

γ -2:4-Dimethoxyphenyl- γ -ketobutyric Acid.—The above ester (28 g.) was refluxed for 3 hours with caustic potash (10%, 150 c.c.). The alkaline solution on acidification gave the keto-acid. It was crystallised from water, m.p. 147°, yield 23 g. (Found: C, 60.33; H, 6.05. $C_{12}H_{14}O_5$ requires C, 60.50, H, 5.88 per cent). The *semicarbazone* was crystallised from

glacial acetic acid, m.p. 166° . (Found : N, 23.92. $C_{13}H_{17}O_5N_3$ requires N, 14.23 per cent).

γ -2:4-Dimethoxyphenylbutyric Acid.—The keto-acid (30 g.) was heated for 9 hours with amalgamated zinc (90 g.) and hydrochloric acid. The product was extracted with ether, the ethereal solution washed with water and dried, ether removed and the residue distilled at $199-201^{\circ}/5$ mm. It solidified when kept in a vacuum desiccator, yield 18 g. It was crystallised from petroleum ether (b.p. $60-70^{\circ}$), m.p. 49° . (Found : C, 63.92 ; H, 7.16. $C_{12}H_{16}O_4$ requires C, 64.29 ; H, 7.14 per cent).

γ -o-Hydroxyphenyl- γ -ketobutyric acid was prepared from succinic anhydride (20 g.), phenol (21 g.), acetylene tetrachloride (100 c.c.) and anhydrous aluminium chloride (60 g.) as usual. The mixture was finally heated at $130-140^{\circ}$ for 2 hours. Excess of phenol and acetylene tetrachloride was removed by steam distillation after adding acidulated water to the mixture. The crude acid was dissolved in sodium carbonate solution and reprecipitated with hydrochloric acid. It was obtained as colourless crystals from water, m.p. 145° , yield 24 g. It gives violet colouration with ferric chloride. (Found : C, 61.73 ; H, 5.23. $C_{10}H_{10}O_4$ requires C, 61.85 ; H, 5.15 per cent).

γ -o-Hydroxyphenylbutyric Acid.—The above keto-acid (20 g.) was heated for 8 hours with amalgamated zinc (60 g.) and hydrochloric acid. The oily liquid was extracted with ether, the ethereal solution washed with water, dried, ether removed when the residue solidified on keeping in a vacuum desiccator, yield almost theoretical. It was crystallised from ether, m.p. 67° . (Found : C, 66.94 ; H, 6.70. $C_{10}H_{12}O_3$ requires C, 66.66 ; H, 6.66 per cent).

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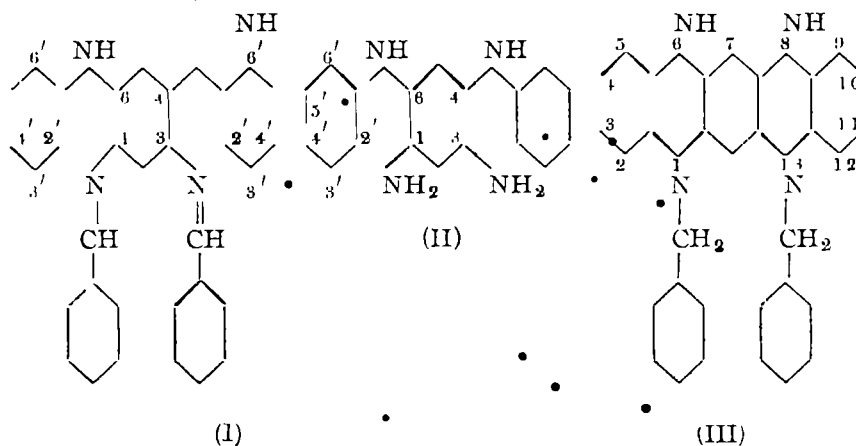
Received December 16, 1938.

ISOMERISATION OF BENZYLIDENE DERIVATIVES. PART I.

By H. S. JOIS, A. KUPPUSAMI AND B. L. MANJUNATH.

The red dibenzylidene compound obtained by condensing benzaldehyde with 4:6-dianilino-1:3-diaminobenzene, when boiled with alcohol, isomerises to a yellowish white substance, which is not hydrolysed by the action of dilute acids. Similarly the benzylidene compounds, prepared by condensing other aromatic aldehydes with the above diamine and with 4:6-*o*-toluidino-1:3-diaminobenzene, undergo isomerisation, whereas the dibenzylidene derivative of 4:6-mesidino-1:2-diaminobenzene remains unchanged. The results are interpreted on the basis of the formation of derivatives of dihydrofluorindene.

It had been observed by Manjunath and Jois (*Proc. Indian Science Congress*, 1930, p. 163) that the red dibenzylidene compound, (I) obtained by condensing benzaldehyde with 4:6-dianilino-1:3-diaminobenzene (II) prepared according to Manjunath (*J. Indian Chem. Soc.*, 1927, **4**, 276), was easily converted on boiling with alcohol to an isomeric colourless compound. This was regarded as due to ring-closure having taken place with the formation of an isomeric heterocyclic compound, 1:3-dibenzylidihydrofluorindene (III).



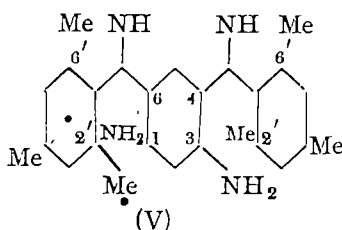
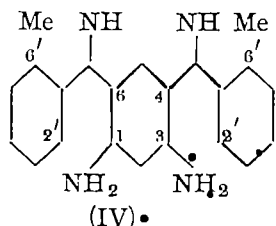
Similar benzylidene compounds were prepared by condensing the amine (II) with salicylaldehyde, anisaldehyde, vanillin, piperonal and cinnamaldehyde.* Each of these could be isomerised to the corresponding dihydrofluorindene derivative. In every case the benzylidene compounds were bright red in colour, while the isomerised compounds were pale yellow

* Attempts to condense (II) with formaldehyde and acetaldehyde under varying conditions resulted in the formation of resinous bodies only.

and exhibited a bluish fluorescence in solution. The red compounds had much lower melting points than their isomers. The benzylidene compounds were easily hydrolysed by acids to give their parent substances but their isomers were quite unaffected on boiling with dilute hydrochloric acid for a long time.

The speed of isomerisation of the different benzylidene derivatives was found to vary considerably. The benzaldehyde derivative isomerises on boiling it with alcohol for an hour or two. The anisaldehyde and cinnamaldehyde derivatives take a slightly longer period. On the other hand, the vanillin and piperonal derivatives isomerise so readily that it is not possible to obtain the benzylidene compounds in a state of purity. This difference in the speed of isomerisation is probably due to the nature and orienting influence of the substituent groups present in the aromatic aldehyde used for condensation.

The benzylidene compounds prepared from 4,6-di-*o*-toluidino-1:3-diaminobenzene (IV) by condensing it with benzaldehyde, salicylaldehyde, vanillin and piperonal also underwent isomerisation. But the benzylidene compounds from 4:6-dimesidino-1:3-diaminobenzene (V) resisted all attempts at isomerisation. From this it follows that one of the two



equivalent positions 2' and 6' in the diamine (II) must be free for the benzylidene compounds to isomerise.

Experiments are in progress to determine the exact nature of the change that takes place during the isomerisations mentioned in this paper.

EXPERIMENTAL.

Dibenzylidene Derivative of (II).—The diamine (II, 1 g.) was dissolved in just the requisite amount of alcohol and while the solution was kept boiling, benzaldehyde (0.73 g.) in alcohol was added. The solution acquired deep red colour and was quickly filtered hot. On cooling beautiful red crystals were obtained which were immediately filtered off and rapidly recrystallised from alcohol as red plates, m.p. 168°. (Found: C, 82.2; H, 5.75; N, 12.0. $C_{32}H_{26}N_4$ requires C, 82.4; H, 5.6; N, 12.0 per cent).

Isomerisation of the Benzylidene Compound (I).—The red compound was dissolved in alcohol and the solution refluxed for 2 hours. The

TABLE I.

Aldehyde used	Properties of the benzylidene compound.	Properties of the isomer.	Mol. formula.	Period required for isomerisation.
Salicylaldehyde	Red prisms, m.p. 204°. (Found: C, 77.1; H, 5.4; N, 11.0%).	Pale yellow needles, m.p. 329°. (Found: C, 77.1; H, 5.3; N, 11.4%).	$C_{12}H_{12}O_2N_4$ requires C, 77.1, H, 5.2, N, 11.2%.	4.5 hrs.
Anisaldehyde	Red prisms, m.p. 176°. (Found: C, 77.7; H, 5.8; N, 10.8%).	Orange yellow crystals, m.p. 294.5°. (Found: C, 77.7; H, 5.6; N, 10.7%).	$C_{14}H_{16}O_2N_4$ " C, 77.6, H, 5.7; N, 10.6%.	3 hrs.
Vanillin	Could not be isolated	Yellowish brown needles, m.p. 313.5°. (Found: C, 73.5; H, 5.4; N, 9.9%).	$C_{14}H_{16}O_4N_4$ " C, 73.4; H, 5.4; N, 10.0%.	Readily isomerises
Piperonal	Crude red material, m.p. 162-69° (isomerises during purification).	Bright yellow needles, m.p. 291°. (Found: C, 73.7; H, 4.9; N, 10.2%).	$C_{14}H_{16}O_4N_4$ " C, 73.6, H, 4.7, N, 10.1%.	Readily isomerises
Cinnamaldehyde	Red prisms, m.p. 184.5°. (Found: C, 83.3; H, 5.8; N, 10.7%).	Yellow plates, m.p. 296°. (Found: C, 83.1; H, 6.0; N, 10.9%).	$C_{16}H_{18}N_4$ " C, 83.2, H, 5.8, N, 10.8%.	3 hrs.

TABLE II.

Benzaldehyde	Red plates, m.p. 247°. (Found: C, 82.6, H, 6.2; N, 11.6%).	Pale yellow needles, m.p. 262°. (Found: C, 82.7; H, 6.2; N, 11.5%).	$C_{14}H_{16}N_4$ " C, 82.6; H, 6.1, N, 11.4%.	3 or 4 hrs.
Salicylaldehyde	Red compound, m.p. 188°. (Found: C, 77.5; H, 5.9; N, 10.7%).	Bright yellow crystals, m.p. 305°. (Found: C, 77.6; H, 5.8, N, 10.8%).	$C_{14}H_{16}O_2N_4$ " C, 77.6, H, 5.7, N, 10.6%.	10-12 hrs.
Vanillin	Could not be isolated	Yellow crystals, m.p. 306°. (Found: C, 73.9; H, 5.9; N, 9.7%).	$C_{16}H_{18}O_4N_4$ " C, 73.7, H, 5.8; N, 9.6%	Readily isomerises.
Piperonal	Red crystals, m.p. 179°. (Found: C, 77.2; H, 4.6; N, 9.7%).	Yellow needles, m.p. 285°. (Found: C, 74.4; H, 4.7; N, 9.7%).	$C_{16}H_{18}O_4N_4$ " C, 74.2; H, 4.5, N, 9.6%.	1 hour.

solution slowly turned yellow and became fluorescent. It was then filtered hot and allowed to cool when pale yellow needles of the isomeric compound (III) separated, m.p. 282.5° . (Found: C, 82.3; H, 5.7; N, 12.1. $C_{32}H_{26}N_4$ requires C, 82.4; H, 5.6; N, 12.0 per cent).

The other benzylidene derivatives of (II) and their isomerisation products were prepared in a similar manner and their properties are given in Table I.

4:6-Di-*o*-toluidino-1:3-dinitrobenzene. — 4:6-Dichloro-1:3-dinitrobenzene (10 g.) was condensed with *o*-toluidine (18 g.) by heating the mixture to 150° for 15 minutes. The excess of the base and its hydrochloride were removed by extraction with dilute hydrochloric acid and then with warm water. The yellow material on crystallisation from alcohol melted at 198° . (Found: N, 14.9. $C_{20}H_{18}O_4N_4$ requires N, 14.8 per cent).

4:6-Di-*o*-toluidino-1:3-diaminobenzene. — The foregoing substance was reduced by refluxing it with boiling sodium hydrosulphide (Manjunath, *loc. cit.*), when the diamine (IV) was obtained as a greyish product. On recrystallisation from xylene it melted at 186° . (Found: C, 75.3; H, 7.1; N, 17.7. $C_{20}H_{22}N_4$ requires C, 75.5; H, 6.9; N, 17.6 per cent).

The amine (IV) was condensed with aromatic aldehydes, as described before, and the properties of the benzylidene derivatives and their isomerisation products are shown in Table II.

4:6-Dimesidino-1:3-dinitrobenzene and 4:6-Dimesidino-1:3-diaminobenzene. — Mesidine (Fittig and Storex, *Annalen*, 1868, 147, 1) was condensed with 4:6-dichloro-1:3-dinitrobenzene to give 4:6-dimesidino-1:3-dinitrobenzene. On recrystallisation from alcohol it was obtained as yellow needles, m.p. 201° . (Found: N, 13.2. $C_{24}H_{26}O_4N_4$ requires N, 12.9 per cent).

It was next reduced with sodium hydrosulphide and the diamine (V), crystallised from alcohol, was found to melt at 152° . (Found: C, 76.9; H, 8.2; N, 15.1. $C_{24}H_{30}N_4$ requires C, 77.0; H, 8.0; N, 15.0 per cent).

Dibenzylidene Derivative of (V). — The diamine (V, 9.5 g.) was condensed with benzaldehyde (3.31 g.) in alcohol when a red crystalline dibenzylidene compound was produced, m.p. 119° . (Found: C, 82.9; H, 7.0; N, 9.9. $C_{38}H_{38}N_4$ requires C, 82.9; H, 6.9; N, 10.2 per cent). It was quite stable and did not isomerise on boiling with alcohol even after 16 hours.

The work, described above, formed part of the thesis submitted by one of us (A. K.) for the degree of Master of Science in the Mysore University and we desire to thank the University for permission to publish the same.

STUDIES ON THE CHANGES OF BLOOD-LIPOIDS OF NORMALLY FED AND VITAMIN C-DEFICIENT GUINEA-PIGS.

BY BAIDYANATH GHOSH.

There is a significant rise in the cholesterol content of blood of guinea-pigs in vitamin C-deficiency.

Recent studies of the changes in the composition of blood and various tissues accompanying the onset of scurvy in guinea-pigs have not revealed striking changes. It has been shown by Ohata (*J. Biochem. Japan*, 1932, **16**, 191) that there is a slight increase in acidity and fatty acid content. Recently, Tislowitz (*Z. Ges. Exper. Med.*, 1935, **97**, 127) has shown that prolonged administration of vitamin C to dogs did not change the blood-cholesterol and he concluded that probably vitamin C has no direct influence on blood-cholesterol metabolism. Dogs, however, can synthesise their own vitamin C and it is likely that their blood ascorbic acid level has a constant value, so that prolonged administration of vitamin C may not appreciably increase this level and consequently any possible influence on blood-cholesterol metabolism by ascorbic acid may not be shown in experiments with dogs. In guinea-pigs, on the other hand, the vitamin C content of blood bears a close relationship to the ingested vitamin until the optimum dose of vitamin C is fed, when naturally the limiting value of ascorbic acid in blood is reached. It has, therefore, been considered desirable to study the effect of vitamin C on the blood lipoids, particularly cholesterol of guinea-pigs.

EXPERIMENTAL.

Male guinea-pigs weighing from 250-350 g. were fed on a basal diet consisting of powdered gram (20 parts), crushed oats (80 parts), salt (1 part), 2-3 drops of cod-liver oil and milk (30 c.c.) per animal, autoclaved for half an hour at 12 lb. pressure. Those receiving this diet alone rapidly declined in weight and developed typical scorbutic symptoms within 3 weeks. Another set of guinea-pigs was fed on germinated gram and green grass in sufficient quantities.

After 3 weeks the blood of the guinea-pigs (of both groups *i.e.*, those that were kept on scorbutic diet and those on normal diet) was drawn out from the heart and a measured quantity (2-3 c.c.) of blood was poured into a 50 c.c. measuring flask containing about 40 c.c. of alcohol-ether (3 : 1) mixture. The flask was gently warmed on a water-bath when it was gently rotated and as soon as the alcohol-ether mixture began to boil, it was removed

and cooled under the tap and finally made up to a definite volume with alcohol-ether mixture. This was filtered and the total lipid (i.e. 3 total fatty acid and cholesterol) was determined from a 20 c.c. aliquot. The alcohol-ether mixture was then saponified with sodium ethylate and evaporated on a water-bath. The last traces of alcohol were driven away by blowing a gentle current of air. The residue was then acidified with dilute sulphuric acid and the lipid matter was extracted by repeated boiling with petroleum ether. The petroleum ether extract was made up to a definite volume from which the total lipid was estimated by evaporation of the petroleum ether and by the chromate oxidation method described by Bloor (*J. Biol. Chem.*, 1928, 77, 53; see also Peters and Van Slyke, "Quantitative Clinical Chemistry" 1932, Vol. 2, 496). The results obtained are given in Table I.

The cholesterol content of the blood was determined from the same alcohol-ether extract. 10 C.c. of the alcohol-ether extract were evaporated to dryness on a water-bath; the dirty residue was extracted by boiling with 3 successive portions of chloroform (1 c.c. each). The extract was decanted to a glass stoppered graduated cylinder. The solution was cooled and was made up to 5 c.c. by addition of chloroform. To the chloroform solution prepared (5 c.c.) acetic anhydride (1 c.c.) and concentrated sulphuric acid (0.1 c.c.) were added. In another similar cylinder a standard solution of cholesterol in chloroform (5 c.c.) was placed and acetic anhydride (1 c.c.) and concentrated sulphuric acid (0.1 c.c.) were added. The contents were thoroughly mixed and kept for 15 minutes to develop colour, which was compared in a colorimeter (Bloor, *J. Biol. Chem.*, 1922, 82, 191; Peters and Van Slyke, *loc. cit.*). The values of cholesterol content of blood are also shown in Table I.

TABLE I.

Guinea-pigs kept on scorbutic diet for 3 weeks.			Guinea-pigs kept on normal diet.		
Mg. per 100 c.c. of blood:			Mg. per 100 c.c. of blood.		
Wt. of animals before drawing blood.	Total lipoids.	Cholesterol	Wt. of animals before drawing blood.	Total lipoids.	Cholesterol.
214 g.	662.5	106	312 g.	625.0	58
189	562.5	90	405	687.5	58
244	500.0	83	350	506.3	60
260	675.0	74	350	506.3	80
265	421.8	80	420	599.5	62
299	875.0	80	400	687.5	60
264	675.0	113	390	537.5	66
222	500.6	121	360	450.0	90
284	675.0	117	440	562.5	66
Mean	616.3	96	Mean	573.4	66

Although the animals show fairly considerable individual variations with reference to the cholesterol and total lipid content of blood, it seems definite that in vitamin C-deficient guinea-pigs there is a rise of cholesterol content in blood, the average rise being of the order of 50%. It, therefore, seems possible that vitamin C has some influence on the cholesterol metabolism of blood. Taking the difference between the total lipoids and the cholesterol content as a measure of the total fatty acid content of blood it appears that this suffers no significant variation being $616.3-96=520.3$ in vitamin C-deficient and $573.4-66=507.4$ in normal condition.

My thanks are due to Prof. B. C. Guha for his advice and interest and to the Indian Research Fund Association for grants.

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CALCUTTA.

Received December 12, 1938.

REVIEW

Newer Methods of Volumetric Chemical Analysis. By ENNA BRENNER, FRESSENIUS CHEMICAL LABORATORY, WIESBADEN; N. HOWELL FURMAN, PRINCETON UNIVERSITY; HELLMUTH STAMM, UNIVERSITY OF HALL; RUDOLF LANG, TECHNICAL SCHOOL, BRUNN AND KASIMIR FAJANS, UNIVERSITY OF MICHIGAN. EDITED BY WILHELM BOTTGGER.

Translated by Ralph E. Oesper, Assistant Professor of Analytical Chemistry, University of Cincinnati. Published by Chapman and Hall Ltd London, 1938. Price 18 sh. 6d. Pp. XIII + 268.

The present volume is a translation of its German original constituting one of the members of the series "Die chemische Analyse" edited by Prof. Wilhelm Bottger of Leipzig. The book is divided into seven parts written by five different authors. These parts deal with:

1. Elimination of titration errors in acidimetric and alkalimetric titrations.
2. Ceric sulphate as a volumetric oxidising agent.
3. Alkaline permanganate solution as a volumetric oxidising agent.
4. Iodate and bromate methods including bromometric method.
5. Chromous solutions as volumetric reducing agents.
6. Oxidation-reduction indicators.
7. Adsorption indicators for precipitation titrations.

Each part of the book is dealt with as thoroughly as possible within its limited space and the methods selected are those which are likely to be very useful in industrial laboratories, where well-tested rapid methods are in great demand. Each topic has been treated both from the theoretical and practical standpoints. A clear exposition of the theory underlying the methods is followed by the presentation of an exact set of directions for the performance of particular analysis. The treatment in each case is critical which would enable the reader to select the best possible procedure for his purpose. The last chapter on Adsorption Indicators by Prof. Fajans contains a great deal of unpublished work by himself and his co-workers.

The value of the book is immensely enhanced as each chapter is supplemented by a comprehensive and up-to-date bibliography.

The book will undoubtedly prove very useful to all research workers and advanced students in analytical chemistry and especially those working in the Industrial laboratories.

The translation, on the whole, has been very good. Mistakes are very rare and almost unrecognisable. The reviewer could detect only very few mistakes in the book. These are:

- (1) the equation on p. 40 is not correct;
- (2) on p. 70, l. 11 from the bottom in place of "summation of (2), (3) and (4) etc." it should be "summation of (3) and (4)" etc.
- (3) on p. 79, l. 10 instead of "iodide" it should read "iodate."

The publisher also deserves congratulation on the excellent printing and get-up of the book.

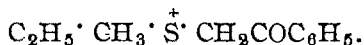
P. R.

THE PRODUCTION OF OPTICALLY ACTIVE SUBSTANCES AND METALLIC FILMS OF SILVER, PLATINUM AND PALLADIUM BY MEANS OF CIRCULARLY POLARISED LIGHT.*

BY PROF. J. C. GHOSH, D.Sc., F.N.I.

In 1824 Fresnel showed that two oppositely circularly polarised light of the same amplitude might not travel through a refracting medium with the same velocity. On passing through such a medium, a plane polarised beam of light, which can be resolved into two such circular vibrations, will show a rotation of the plane of polarisation. Pasteur showed that in transparent crystals, such rotatory powers are associated with hemihedral faces which are so oriented that the *dextro* crystal is the mirror image of the *laevo* one. This kind of asymmetry is not confined to crystalline structures only. Molecules themselves may be asymmetric, and in solution or the gaseous state, can rotate the plane of polarisation of light. Van't Hoff arranged the atoms of the chemical molecule in three dimensions and directed the four valences of the carbon atom towards the corners of a tetrahedron with the carbon atom at its mass centre. If all the four atoms or radicals attached to the central carbon atom are different, optical isomerism will arise, one configuration being the mirror image of another and not superposable. Van't Hoff also foreshadowed the existence of dissymmetric compounds of the allene type which would show optical activity even in the absence of an asymmetric central atom. In the case of complex co-ordination compounds containing six radicals round a central metal atom, Werner postulated an octahedral model, and demonstrated the conditions which would produce optical isomerism. The list of elements from which optically active compounds have been prepared has now been extended to twenty-one elements as follows :—

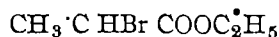
Be, B, C, N, Al, Si, P, S, Cr, Fe, Co, Ni, Cu, Zn, As, Se, Te, Ru, Rh, Ir and Pt. In preparing these compounds no fundamentally new principles have been discovered other than those postulated by Van't Hoff and Werner excepting perhaps the significant one, that a lone pair of electrons in an octet can take the place of a radical in bringing about optical isomerism in ions of the type



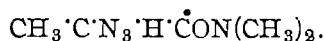
* Presidential address delivered at the Annual General Meeting of the Indian Chemical Society, at Lahore on Jan. 7, 1909.

In synthetic work in the laboratory, racemic mixtures are always obtained consisting of exactly equal proportions of the two optical isomers. Pasteur's original method of resolving these racemic mixtures by the use of optically active acids or bases has been considerably extended by Pope and others in recent times, and it is now possible to effect resolution for any such mixture.

In living organisms, however, only one type of optically active molecule is produced, and the artificial resolution of racemic compounds in laboratories is effected by optically active substances which ultimately owe their origin to natural sources. Ever since Wöhler's synthesis of urea, the upholders of the vitalistic theory have continually retreated from their untenable positions until they held on to the production of optically active compounds as the chief plank in their support. As early as 1894, Van't Hoff remarked that it is exceedingly probable that under asymmetric conditions of experiment, the same direct formation of optically active bodies will result as we observe in the processes of natural synthesis, *e.g.*, in transformations taking place under the action of *dextro* or *laevo* circularly polarised light. It is only, however, almost a century after the classical work of Wohler, that in 1930 Kuhn and collaborators succeeded in decomposing a solution of



in alcohol with the aid of circularly polarised light ($\lambda=2800\text{\AA}$). It was found that if 50% of the ester were decomposed with the aid of *dextro*-light, the solution that remained over, was found to show *dextro*-rotation indicating that under the influence of *dextro*-light in the ultraviolet, the *laevo*-ester decomposed much faster than the *dextro*-ester. The next important investigation was that of Freudenburg and Kuhn where the following ester was used :—



Hexane solutions of this compound were exposed to *laevo* rotations ($\lambda=2800\text{\AA}$). After 37% decomposition by *laevo*-light, the unchanged dimethylamide was separated from hexane and other decomposition products by distillation under reduced pressure. It showed a rotation of, 1° in a decimeter tube in mercury yellow light.

It may be asked that these experiments are not very difficult—why then was there a long tale of frustrated attempts between Van't Hoff's prophecy of 1894 and Kuhn's achievement in 1930. I shall try to indicate the explanation

in simple language. The rotatory power of an optically active substance is generally measured for the sodium D line, but this power changes with wave-length much in the same way as does the refractive index of a medium. Indeed, Drude long ago derived an equation for rotatory dispersion of the form

$$\alpha = \frac{D}{\lambda^2 - \lambda_m^2}$$

where α is the observed rotation at wave-length λ , λ_m is the wave-length corresponding to a characteristic frequency of vibration in the neighbourhood of an absorption band caused by the vibration of an asymmetric electronic band, and D is a constant characteristic of the molecule. Thus for tartaric acid in aqueous solution,

$$\alpha = \frac{A}{\lambda^2 - 0.03} - \frac{B}{\lambda^2 - 0.054} \theta$$

For $\lambda_m^2 = 0.054$, $\lambda_m = 2330 \text{ \AA}$ which is close to an absorption band in the ultraviolet for tartaric acid. For $\lambda_m^2 = 0.03$, λ_m lies far in the Schumann region.

Besides rotating the plane of polarisation, optically active molecules may possess another property — circular dichroism, *i.e.*, the absorption of *dextro*-light may be different from that of *laevo*-light. On emergence from a medium, which is both optically active and circularly dichroic, the plane polarised light becomes not only less intense, but it also becomes elliptically polarised, with the major axis of the ellipse inclined to the original plane of polarisation at an angle, which for small ellipticities, we may also call, the rotation of the plane of polarisation. Optical instruments of considerable sensitiveness have been devised by Bruhat for measuring such ellipticities and rotation.

Modern theories of photochemical reactions postulate that the amount of photochemical transformation is proportional to the number of light quanta absorbed by the reacting molecules. If a racemic mixture is illuminated by *dextro*-light, and the dichroism is such that the *dextro*-molecules absorb more of the *dextro*-light than the *laevo*-molecules, then it is expected that the rate of transformation of *dextro*-molecules will be faster than that of *laevo*-molecules. The result will be, that if the reaction proceeds, the optically inactive racemic mixture will show increasing *laevo*-activity. The essential conditions for success are however the following :—

1. The radiations used for photochemical transformation should correspond with the absorption band, which is characteristic of the asymmetric electronic bonding as indicated by Drude's equation of rotatory dispersion.
2. The substance should exhibit circular dichroism in the neighbourhood of the band head.

Kuhn's success was due to the fact that the propionic acid derivatives, which he used, gave a rotatory dispersion which indicated a characteristic band in the neighbourhood of 2800 \AA and that the absorption of *dextro*-light of this wave-length by *dextro*-molecules was different from that of *laevo*-molecules.

In the plant kingdom, the process of photosynthesis takes place with the aid of catalytic agencies like chlorophyll, xanthophyll, etc. The exact mechanism by which they produce complex organic molecules from carbon dioxide and water with the aid of the visible radiations from the sun, is even now a subject for considerable speculation. It is found that the complex molecules produced as a result of photosynthesis, are either *dextro*-rotatory or *laevo*-rotatory, if they contain one or more asymmetric carbon atom. Experiments now in progress in our laboratories indicate that an acetone solution of one of the photocatalysts, chlorophyll-*a*, is both optically active and circularly dichroic.

TABLE I.

Measurement of rotatory dispersion and ellipticity of chlorophyll-*a* in acetone solution.

($M/20,000$)

λ .	Length of solution.	Rotation in degrees.	Ellipticity in degrees.
6700	1.5 cm.	-0.04	+0.04
6200	2.5	-0.1	+0.05
5780	4.0	-0.01	+0.00
5460	8.0	-0.05	+0.04
4916	5.0	-0.04	+0.07

It has also been possible to prepare colloidal solution of inorganic catalysts which are circularly dichroic. Thus, if we take dilute solutions of sodium tungstate and hydrochloric acid and immediately after mixing them together, expose them to powerful *laevo*-circularly polarised ultraviolet radiations (between $3100\text{--}3600 \text{ \AA}$), the molecules during the process of aggregation to form colloidal micelles appear to be subjected to the directive

influence of such radiation. Table II gives the values of circular dichroism which have been observed for such colloidal solutions.

TABLE II.

Induced anisotropy of photo-sensitive sols in 366 μ .

Substance studied.	Conc.	Period of pre-excitation (Intensity of exciting radiation incident = 950 ergs per sq. cm. per sec.).	Nature of exciting radiation	Photometric method.	*Thermopile-electrometric method.
$g = \frac{\epsilon_s - \epsilon_d}{\epsilon_s} \quad g = \frac{\epsilon_1 - \epsilon_2}{\epsilon_s}$					
1. Tungstic acid	0.0125 M (in terms of tungstate)	6 hours	l-Circularly polarised	+0.0271	+0.016
"	0.0125	6	d-Circularly polarised	-0.0221	-0.012
"	0.0125	...	Kept in the dark	+0.0001	Nil
2. Ceric borate	0.0036	12	l-Circularly polarised.	...	+0.0246
"	"	"	d-Circularly polarised.	...	-0.0257
"	"	...	Kept in the dark for 12 hrs.	...	Nil
3 Vanadic acid	0.0025 M (in terms of vanadate)	10	l-Circularly polarised	+0.0289	Nil
"	0.003	12	"	...	+0.013
"	0.0025	10	d-Circularly polarised	-0.0054	Nil
"	0.003	12	"	...	-0.0084 -0.0084 -0.008
			Kept in the dark	+0.0087	Nil

TABLE II (contd.).

Thickness of the cell = 0.2 cm.

Substance studied.	Conc.	Period of pre-excitation.	Nature of the exciting radiation	Photometric method.	**Thermopile-electrometric method.
4. Chromic tungstate	0.02 M (in terms of tung. state).	10 hrs.	L-Circularly polarised	—	+0.0227
		10	d-Circularly polarised	-0.0284	-0.0296
			Kept in the dark	-0.0067	Nil.

A colloidal solution of tungstic acid does not reduce glucose in the dark. But in presence of light, the glucose is oxidised and tungstic acid is reduced to form a blue colloidal solution. The velocity of this photochemical reaction has been carefully studied. A colloidal solution of tungstic acid is taken in which circular dichroism has been developed by maturing it in *laevo*-light, mixed with a solution of glucose, and then the velocity of photochemical reaction, studied under the influence of *dextro*- or *laevo*-light ($\lambda = 3660\text{\AA}$) of the same intensity. It is found that the velocities are not identical.

TABLE III.

The reaction was carried in the ultraviolet (3660\AA) K_0 , the zero-molecular velocity constant, denotes the number of g. mol. transformed per unit cell per second.

Sol. pre-activated in L-circularly polarised light for 6 hours, the intensity of the exciting light being 113 ergs.	Reaction carried in	I_{abs} in ergs in which the reaction was carried.	$K_0 \times 10^{10}$.
	L-Circularly polarised light.	113	0.93
	d-Circularly polarised light.	113	0.806

* The photometric method has been described (J. Indian Chem. Soc., 1937, 15, 502-518). The thermopile-electrometric method will be described in a paper to be published shortly.

A photocatalytic dichroic system for visible radiations can be obtained by reducing in ordinary light a mixture of sodium vanadate and excess of *dextro*-tartaric acid. If we take a solution of sodium vanadate and add *dextro*-tartaric acid in excess (final concentrations:—0.05 M-NaVO₃ and 0.08M-tartaric acid) the solution is coloured red due to the formation of a complex of vanadic acid sol with *d*-tartaric acid. The solution is not circularly dichroic. If, however, it is exposed to ordinary light until the whole of the vandate (V⁵) has been reduced to the quadrivalent (V⁴) stage, a violet coloured solution is obtained which is both circularly dichroic and optically active. The rotatory dispersion and ellipticities of such solutions are given in Tables IV and V.

TABLE IV.

Mixtures containing d-H₂T complex.

E—ve is right-handed. *E*+ve is left-handed. Conc. same as in Table I.

Wave-length.	Length of solution.	Rotation.	Ellipticity (<i>E</i>).	Mol. extn. coeff.	Anisotropy factor (<i>g</i>).*
6700Å	3 cm.	+0.18°	-0.25°	13.68	-0.00739
6200	6	+0.66	-0.14	13.03	-0.00218
5780	6	+0.48	-0.09	12.38	-0.00147
5460	6	+0.30	-0.04	11.74	-0.00069
5200	6	+0.26	-0.06	11.10	-0.00109
4916	6	+0.16	+0.14	9.83	+0.00288
4358	6	+0.10	+0.14	8.58	+0.00288

TABLE V.

Mixture containing l-H₂T complex.

Concentration same as in Table I.

Wave-length.	Length of solution.	Rotation.	Ellipticity (<i>E</i>).	Mol. extn. coeff.	Anisotropy factor (<i>g</i>).*
6700Å	3 cm.	-0.20°	+0.50°	13.68	+0.0148
6200	6	-0.50	+0.30	13.03	+0.00467
5780	6	-0.35	+0.20	12.38	+0.00327
5460	6	-0.30	+0.23	11.74	+0.00397
5200	6	-0.27	+0.22	11.10	+0.00400
4916	6	-0.25	-0.05	9.83	-0.00103
4358	6	-0.24	—	8.58	—

* $g = \frac{\text{Extinction coefficient for } l\text{-light} - \text{Extinction coefficient for } d\text{-light}}{\text{Extinction coefficient for ordinary light}}$

It will be noticed that with *l*-tartaric acid, similar results but in the opposite sense are obtained both for circular dichroism and rotatory dispersion.

By the use of a solution of potassium persulphate as an oxidant, it is possible to oxidise tartaric acid with this reduced vanadium complex as a photo-catalyst. The reaction is zero-molecular in light and does not take place in the dark. Velocities are given in Table VI.

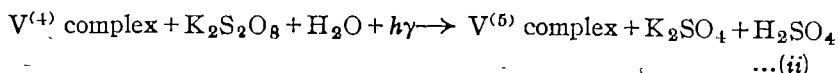
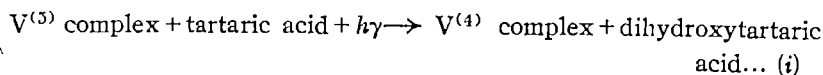
TABLE VI.

Concentration of vanadium in the reduced vanadium tartaric acid complex = 0.025 *M*. Concentration of $K_2S_2O_8$ = 0.05 *M*. Temperature = 25°. $I_{abs} = 1384$ ergs (in all cases)

Light.	$\frac{dx}{dt} \times 10^5$ for				
	Reduced complex of <i>d</i> -H ₂ T.	<i>l</i> -H ₂ T.	<i>dl</i> -H ₂ T.*	Reduced complex of <i>r</i> -H ₂ T.	
<i>d</i> -Light	2.71	Mean 1.29	1.87	2.3	2.3
<i>l</i> -Light	1.87	Mean 2.29	2.71	2.3	2.3

Quantum efficiency of the reaction in circularly polarised light varies from 0.247 to 0.358. For *dextro*-light, the reduced vanadium complex of *d* tartaric acid has a greater extinction coefficient and the quantum efficiency in *dextro*-light is greater than that in *laevo*-light. The reverse is the case when *laevo*-light is used for the oxidation of the reduced complex of vanadium with *l*-tartaric acid. A larger fraction of the incident light is absorbed by the surface layers of the colloidal micelles when the extinction coefficient is larger, and since the chemical reaction mostly takes place on such surface layers, the quantum efficiency is expected to increase.

If tartaric acid is present in excess, the following photo-catalytic cycle continues until the whole of the persulphate is reduced.



* *dl*-H₂T means mixtures of *dextro*-tartaric and *laevo* tartaric acid in equivalent proportions.

The $V^{(5)}$ complex is not circularly dichroic and the velocity of reaction (i) is independent of the nature of polarisation of the photoactive radiations. For reaction (ii), as we have already seen in Table VI, the observed velocity in *dextro*-light, when racemic tartaric acid is used for complex formation and photo-reduction of persulphate, will be the mean of the velocities observed for reactions with *dextro*-tartaric acid and *laevo*-tartaric acid taken separately. With racemic acid, the initial rotation is zero, but with the progress of oxidation in *dextro*-light, *d*-tartaric acid will be oxidised faster than *l*-tartaric acid and the residual mixture will develop *laevo*-rotation. Table VII shows that this is found to be the case.

TABLE VII.

Length of solution = 2.5 cm. Time of exposure = 8 hours.

Wave-length = 5890 Å. Concentration same as in Table I.

Light.	Observed rotation.		
	<i>dl</i> -H ₂ T-complex.	<i>rr</i> -H ₂ T-complex.	Calculated value (for <i>dl</i> -H ₂ T or <i>rr</i> -H ₂ T).
<i>d</i> -Circular	-0.06°	-0.06°	-0.061°
<i>l</i> -Circular	+0.06	+0.06	+0.061

In Table VII the calculated value for the expected rotation is given by

Rotation (calculated) =

$$\mp \frac{\text{Change in rotation on oxidation}}{\text{Change in titre}} \times \frac{a-b}{a+b} \times c.$$

for *dextro* and *laevo*-light respectively where the ratio $\frac{\text{change in rotation}}{\text{change in titre}}$ is a constant which is pre-determined for mixtures of $K_2S_2O_8$ and *d*-H₂T or *l*-H₂T complex, *a* and *b* are the velocities for reaction in *dextro*- and *laevo*-light respectively (Table VI) and *c* is the actual change in titre observed immediately after the rotation, as given in Table VII, has been measured. There is good agreement between the observed and the calculated values,

Production of Metal Films which are Optically Active and Circularly Dichroic.

It is well known that enzymes yield end-products which are always optically active if they contain one or more asymmetric carbon atom. Take for instance fumaric acid. By addition of water, it can produce malic acid, and by addition of ammonia, aspartic acid. Enzymes from muscle extract or *B. coli* often produce *l*-malic acid and *d*-aspartic acid. Enzymes are colloidal aggregates and it is probable that when produced inside living organisms, they are endowed with some kind of asymmetric structure. Early in the beginning of this century Bredig found that colloidal platinum behaves, for purposes of oxidation and reduction, like enzymes, and he gave such sols the name of inorganic enzymes. But of course they do not simulate the enzymes in the matter of producing optically active substances. We have been for some time trying to produce films of catalytically active metals like silver, platinum and palladium with definite asymmetric structure so that they may exhibit both optical rotation and circular dichroism. The results that have been obtained are such as to encourage the hope that asymmetric synthesis analogous to that brought about by enzymes, may be effected with such asymmetric films of inorganic metal catalysts.

The pioneering work of Weigert gave the clue to such investigations. A glass plate is covered with a thin layer of a suspension of silver chloride in gelatin. It is dried and exposed to normal light till the colour turns bluish red—photochloride is formed. This layer is then exposed to strong plane polarised red light from an arc-lamp. The red spot produced is found to be dichroic. Spots produced by vertical vibration have far greater transmission for vertical vibrations and the same is true of horizontal vibrations. Various theories have been proposed for this Weigert effect but without entering into their merits, we shall recognise the fact that the photomicrospheres are arranged with their axis parallel to the direction of vibrating light which is used for excitation. It is natural to expect that circularly polarised light will bring about such a circular arrangement of the photomicrospheres of AgCl, and some preliminary experiments on this point were reported by Zocher about a decade ago.

Gelatin is an optically active material, and if optical rotation and circular dichroism are to be developed in photomicrospheres in films of AgCl, gelatin as a supporting material has got to be altogether excluded; and very thin glass discs free from all strain can only be used. A film of AgCl

on such glass in absence of gelatin is extremely delicate, being often washed off in contact with water. Adhesive films of AgCl, after many fruitless efforts, have been prepared in our laboratories according to the following technique.

Microscopic cover-discs, free from ellipticity, were cleaned with chromic acid, alkaline solution, water and absolute alcohol and dried. Silver mirror was deposited on these films by the evaporation of a pure silver wire placed between two tungsten electrodes in a chamber evacuated to 10^{-6} mm. The presence of Hg vapour in the chamber was excluded by traps of copper gauge. The silver mirrors were converted into silver chloride films by placing them above chlorine water in a closed chamber. After complete chlorination, the discs were immersed in purest distilled water and in successful experiments AgCl film was often found to adhere to the glass surface. This moist film was then exposed to the ordinary light of mercury arc until by the production of photomicrospheres a violet colour was obtained. If such a violet film is next exposed for 30 minutes to *dextro*-light from a carbon arc at 6 amperes, it develops both optical activity and circular dichroism.

Rotation at 6700 Å	-0.1°
Ellipticity	+0.04

The thickness of the silver film was determined by a method of microchemical analysis developed in Kodak laboratory and was of the order of 0.3×10^{-4} cm., i.e., half of the wave-length of red light. It means that calculated on the basis of 1 cm. thickness, the film would show the extraordinary rotation of -10.000° .

The AgCl film can be developed either physically or chemically by the oxalate developer or rhodinal developer. The resulting film of silver gave

Rotation	-0.06°
Ellipticity	+0.04

The silver film can be toned with platinum and the resulting platinum film gave

Rotation	-0.1
Ellipticity	+0.15

Such platinum films have been tested as regards complete substitution of silver by platinum by the action of nitric acid, the rotation and ellipticity remaining unchanged even under such drastic changes.

Toning with palladium also gives similar results.

Investigations on the physical properties of these films and the catalytic action of these films with the object of producing optically active substances are now in progress.

My thanks are due to my collaborators Dr. T. Banerjee, Dr. B. C. Kar and Messrs T. L. Ramachar, K. R. Kar, and my colleague Mr. S. K. Mukherjee for much of the experimental work recorded in this paper.

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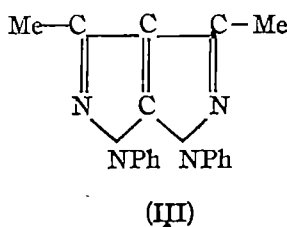
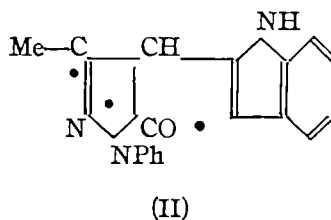
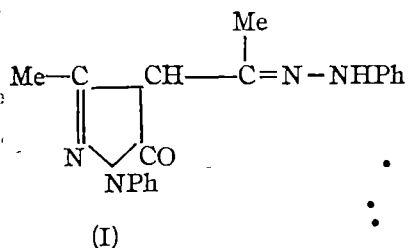
• PYRAZOLE DERIVATIVES.

BY TEJENDRA NATH GHOSH AND DEBABRATA DAS-GUPTA.

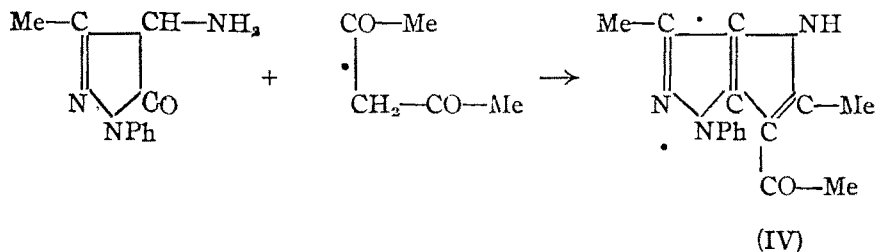
An indolylpyrazolone derivative has been synthesised. The syntheses of some pyrrolopyrazole and dipyrazole derivatives have been described.

The synthesis of pyrroloquinol has been described by Mrs. Robinson (*J. Chem. Soc.*, 1929, 2948) and of pyrrolyndoles by Aggarwal, Qureshi and Ray (*J. Amer. Chem. Soc.*, 1932, **54**, 3988). In view of the fact that some pyrazolone derivatives (*e.g.* antipyrine, amidopyrine, etc.) exhibit pronounced antipyretic properties, it seemed of interest to synthesise some indolylpyrazolone derivatives.

3-Carboxylimino-4-acetylphenylhydrazone-1-phenylpyrazolone (Ghosh, *J. Indian Chem. Soc.*, 1936, **13**, 87) could not be successfully made to undergo the Fischer indole transformation. However, the phenylhydrazone (I) of 1-phenyl-3-methyl-4-acetylpyrazolone has now been transformed to α -indolyl pyrazolone derivative (II) with zinc chloride. Alcoholic hydrochloric acid transforms the compound (I) to the dipyrazole derivative (III). This indicates that phenylhydrazone derivatives of the type (I) require drastic conditions for conversion into indoles (*cf.* Brunck, *Annalen*, 1893, **272**, 201; Ray *et al.*, *loc. cit.*; Mumm and Petzold, *Annalen*, 1938, **536**, 23).



1-Phenyl-3-methyl-4-aminopyrazolone condenses with acetylacetone to furnish the pyrrolopyrazole derivative (IV) (cf. Duden, *Annalen*, 1900, 318, 25; Dohrn and Thiele, *Ber.*, 1931, 64, 2863). The compound (IV) reacts with benzaldehyde to give a chalkone.



EXPERIMENTAL.

3-Methyl-4-acetylphenylhydrazone-1-phenylpyrazolone (I), prepared by adding an equimolecular proportion of phenylhydrazine to an alcoholic solution of 1-phenyl-3-methyl-4-acetylpyrazolone (Stolz, *J. pr. Chem.*, 1897, 55, 154), was crystallised from alcohol in colourless slender needles, m.p. 197°.

4-a-Indolyl-1-phenyl-3-methylpyrazolone (II).—An intimate mixture of the compound (I, 12 g.) and anhydrous zinc chloride (50 g.) was heated at 200° for 3 hours. The product was cooled, powdered and thoroughly washed with dilute hydrochloric acid and hot water. The solid obtained was found to contain zinc. It was, therefore, kept in contact with cold concentrated hydrochloric acid for about 1 hour and filtered. The clear acid solution, on dilution with water, gave a brown pasty mass which, however, could not be purified. The insoluble portion was extracted with glacial acetic acid; the solution, on dilution with water, furnished a solid (3 g.) which was further purified by dissolving in cold dilute alkali and precipitating with hydrochloric acid. It was finally crystallised from alcohol (charcoal) in colourless shining rectangular plates and dried at 140°, m. p. 238° (decomp., shrinking at 225°). (Found: C, 74.42; H, 5.28; N, 14.27. $\text{C}_{18}\text{H}_{16}\text{ON}_3$ requires C, 74.74; H, 5.19; N, 14.53 per cent). It is readily soluble in cold dilute alkali, from which it is precipitated by acid as a white flocculent mass. Its alcoholic solution gives a red colouration with ferric chloride. A solution of the substance in concentrated sulphuric acid gives a deep green colour with alloxan. With isatin and sulphuric acid the red colour, slowly changes to deep violet. If a minute quantity of the substance is added to an alcoholic solution

of a small quantity of *p*-dimethylaminobenzaldehyde and 2-3 drops of dilute hydrochloric acid, and the solution is boiled for about 5 minutes and cooled, a light pink colour is developed. If to this solution 2 drops of concentrated hydrochloric acid are now added and the solution heated for about 1 minute, the colour changes to light green.

1:1'-Diphenyl-3:3'-dimethyl-(4:5:4':5')-dipyrâzole (III).—The compound (1.6 g.) was dissolved in absolute alcohol (50 c. c.), saturated with dry hydrogen chloride, when a hydrochloride was deposited as a voluminous precipitate. The hydrochloride went into solution on heating for 6 hours. Alcohol was removed in *vacuo* and the product treated with sodium carbonate solution, when a pasty mass was obtained which soon solidified. The brown solid (3.5 g.) was first crystallised from a mixture of alcohol and water, and then from hot water (charcoal) in colourless rectangular plates, m. p. 129-30° (softening at 123°). (Found: C, 70.27; H, 6.42; N, 18.13, 18.33; H₂O, 5.42. C₁₈H₁₆N₄, H₂O requires C, 70.59; H, 5.88; N, 18.30; H₂O, 5.88 per cent). The molecule of water is tenaciously held and only removed completely, when the substance is heated at 140-142° in vacuum. After melting, the substance remains as a colourless syrupy mass, which does not solidify even on standing for several days.

Whereas the compound (I) is soluble in cold dilute alkali and gives a deep red colouration with ferric chloride, the substance (III) is insoluble in alkali and does not give any colouration with ferric chloride. It is soluble in cold concentrated hydrochloric acid and is not precipitated on dilution with water. The acid solution, on evaporation under reduced pressure, gave a syrupy mass soluble in cold water, which did not solidify even on long standing in vacuum. The hydrochloride is soluble in alcohol and is precipitated as an oil by addition of ether. The substance (III) does not contain any diazotisable amino group.

1-Phenyl-3-methyl-4:5-(2'-methyl-3'-acetopyrrolo) (4':5')-pyrazole (IV).—To a glacial acetic acid solution of 1-phenyl-3-methyl-4-aminopyrazolone, prepared according to Knorr (*Annalen*, 1887, 238, 158, 189) an equimolecular proportion of acetylacetone was added and the solution was heated on the water-bath for about 6 hours. The solution, which was orange-coloured at the beginning turned deep red on heating. It was cooled and diluted with water, when a reddish brown solid separated which was washed with alcohol and ether. It is practically insoluble in hot alcohol and crystallised from large quantity of glacial acetic acid in colourless rectangular plates, m. p. above 320°, yield about 5%.

(Found: C, 70.82; H, 6.33, N, 16.49. $C_{15}H_{15}ON_3$ requires C, 71.14; H, 5.92; N, 16.60 per cent). It is readily soluble in cold dilute alkali and precipitated by acid. Its solution in glacial acetic acid does not give any colouration with ferric chloride. With cold concentrated hydrochloric acid it forms the hydrochloride as colourless powder, which is readily hydrolysed by water. With concentrated sulphuric acid the substance turns red and a dark-coloured solution is obtained. A minute quantity of the substance in an alcoholic solution of traces of *p*-dimethylaminobenzaldehyde and 2-3 drops of concentrated hydrochloric acid, gave on heating a pale yellow clear solution which when cooled and treated with sodium nitrate, developed a fine bluish green colour.

The yield of the compound (IV) was increased to about 10% by carrying out the above reaction in glacial acetic acid in presence of hydrogen.

The benzylidene derivative was obtained by heating the above substance for 2-3 hours with equimolecular proportion of benzaldehyde in alcoholic solution containing the requisite quantity of alkali. The solution was cooled and acidified with cold dilute hydrochloric acid. The solid, thus obtained, crystallised from acetic acid in colourless rectangular plates, m. p. above 300°. (Found: N, 12.45. $C_{22}H_{19}ON_3$ requires N. 12.31 per cent). It dissolves in concentrated sulphuric acid to give a deep green solution.

The authors' grateful thanks are due to Professor P. C. Guha for his kind interest in this investigation.

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Received January 13, 1939.

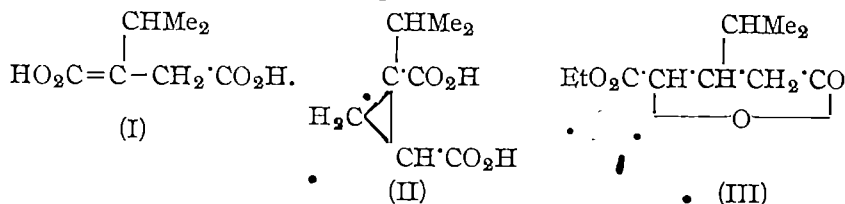
β-isoPROPYLGLUTACONIC ACID.

By S. K. RANGANATHAN

The synthesis of *trans*-β-*isopropyl*glutaconic acid starting from ethyl *isobutyryl* acetate is described. It has been converted into its *cis*-isomer.

The investigation herein described was undertaken in continuation of the work on the synthesis of umbellularic acid (Ranganathan, *J. Indian Chem. Soc.*, 1936, 13, 419). It was thought that β-*isopropyl*glutaconic acid (I) would form a suitable intermediate for the synthesis of *thujad*-dicarboxylic acid (II), direct evidence for the presence of *cyclopropane* ring in the latter is still unestablished. β-*iso*Propylglutaconic acid has now been synthesised, but however, due to adverse yields throughout the entire process, it has not been found possible to utilise it for the object contemplated.

Gibson and Simonsen (*J. Chem. Soc.*, 1929, 1074) attempted to synthesise β-*isopropyl*glutaconic acid by the elimination of hydrogen bromide from ethyl α-bromo-β-*isopropyl*glutarate with diethylaniline. The main product of the reaction, however, was the lactonic ester (III), no trace of either an unsaturated or a cyclic ester being formed.

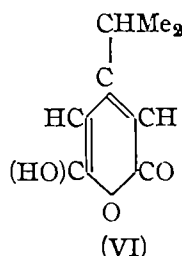
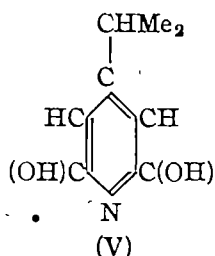
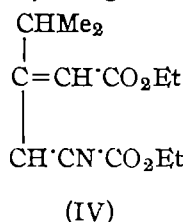


Ethyl β-hydroxy-β-*isopropyl*glutarate could not be used for the synthesis as it could not be prepared (*cf.* Gibson and Simonsen, *loc. cit.*). In the present investigation we have adopted the method of Rogerson and Thorpe (*J. Chem. Soc.*, 1905, 87, 1685) for the synthesis of the requisite acid (I).

A slight modification of the method of Bouveault and Bongert (*Bull. Soc. chim.*, 1902, iii, 27, 1004, 1089) gave the difficultly procurable ethyl *isobutyryl*acetate in workable amounts (*cf.* experimental part). The condensation of sodio-derivative of ethyl cyanoacetate with ethyl *isobutyryl* acetate yielded ethyl α-cyano-β-*isopropyl*glutaconate (IV) in uncertain amounts, the yield being never more than about 5% and at times only a trace being formed. The substitution, however, of potassium for sodium (*cf.* Hope, *J. Chem. Soc.*, 1922, 121, 2216; Kon and Nanji, *ibid.*, 1931, 560) had the effect in raising the yield to a maximum of 20%, subject however, to certain conditions described in the experimental part. On hydrolysis with hydrochloric acid ethyl α-cyano-β-*isopropyl*glutaconate furnished β-*isopropyl*glutaconic acid (I), in about 5-10% yield, along

with the hydrochloride of 2:6-dihydroxy-4-isopropylpyridine (V) (Gibson and Simonsen, *loc. cit.*) which was formed in prepondering amount.

Following Bland and Thorpe (*J. Chem. Soc.*, 1912, 101, 856) the *trans* configuration has been assigned to the above acid. With acetyl chloride the *trans*-acid gave the hydroxy anhydride (VI) which on treatment with 30% potassium hydroxide furnished the *cis*-acid (?). Water decomposed the hydroxy anhydride, however, into the *trans*-acid (*cf.* Bland and Thorpe, *loc. cit.*). The conversion of the *cis*-acid into the *trans*-isomer has not been effected, owing to lack of sufficient material.



Ethyl isobutyrylmalonate (Knoevenagel and Faber, *Ber.*, 1898, 31, 2770) does not condense with ethyl cyanoacetate to yield ethyl α -cyano- β -isopropyl- γ -carbethoxyglutaconate.

EXPERIMENTAL.

isobutyryl Chloride.—A good yield of the substance was obtained by the following method. To the acid (192 g.) cooled at 0°, phosphorus trichloride (200 g.) was added gradually. The mixture was allowed to stand overnight at room temperature, after which it was heated on the water-bath for 2 hours. The acid chloride was decanted from the phosphorus acid and fractionally distilled, b.p. 85-88°/680 mm., yield of twice distilled product being 220 g.

Ethyl isoButyrylacetate.—Some preliminary attempts were made to prepare the substance by the application of the method of Fischer, Goldschmidt and Nussler (*Annalen*, 1931, 486, 31) for the preparation of ethyl *n*-butyrylacetate. The method involves separation and purification from ethyl acetoacetate by fractional distillation. It was, however, found impracticable in the present case, a mixture of ethyl isobutyrylacetate and ethyl acetoacetate being invariably formed, which could not be separated by fractional distillation.

The following process was adopted (*cf.* Bouveault and Bongert, *loc. cit.*). To molecular sodium (46 g.), suspended in anhydrous ether (1 litre), freshly distilled ethyl acetoacetate (160 g.) was added dropwise under cooling. To the sodium salt isobutyryl chloride (213 g.) was added dropwise, care being taken to see that the temperature never

rose above 0° . After the addition, the product was allowed to stand overnight, and was then decomposed with water. The ethereal layer was separated, dried and ether removed from it. The residue was repeatedly extracted with 50-70 c.c. portions of ice-cold 9% sodium hydroxide solution till the extract showed no longer any increase in volume (12 times). The united alkaline extract was once extracted with ether, after which it was decomposed with hydrochloric acid. The oil that separated was extracted with ether, the ethereal solution washed with aqueous sodium bicarbonate and water and then dried and ether removed. The residue was distilled when ethyl *C-isobutyryl*acetoacetate boiled at $100^{\circ}/6$ mm., yield 107 g. This was shaken up with 10% ammonium hydroxide (200 c.c.) for 20 minutes after which the mixture was warmed on the water-bath at 40° for 25 minutes to complete the reaction. The oily layer was extracted with ether, the ethereal solution washed with water, aqueous sodium bicarbonate (2 times), and again with water, then dried and ether removed. The residue was distilled in vacuum. Ethyl *isobutyryl* acetate boiled at $72^{\circ}/3$ mm., yield 70.5 g.

The *semicarbazone*, prepared in the cold, melted at 103° . (Found: N, 19.7. $C_{18}H_{17}O_3N_3$ requires N, 19.5 per cent).

Ethyl α -Cyano- β -isopropylglutaconate (IV).—Ethyl *iso-butyryl*acetate was condensed with ethyl sodiocyanoacetate according to the method of Rogerson and Thorpe (*loc. cit.*). The yield of the ester (IV) was exceedingly low. A cold solution of potassium (13 g.) in absolute alcohol (150 c.c.) was mixed with ethyl cyanoacetate (37.6 g.) with shaking, and the potassium derivative was cooled to 0° and treated with ethyl *isobutyryl*acetate (52 g.) and the whole kept in a freezing mixture for 6 hours and then at room temperature for 3 days with occasional shaking. The potassium derivative of the original ester dissolved and gave place to that of the condensation product. It was decomposed with water, the red coloured solution acidified with hydrochloric acid and the oily layer that separated was taken up in ether. The ethereal solution was washed thoroughly with sodium carbonate solution, water, and ether removed. The residue [*ethyl α -cyano- β -isopropylglutaconate* (IV)] distilled at $145^{\circ}/3$ mm., as a colourless viscid oil, yield 1.3 g. (36 g. of ethyl *isobutyryl* acetate were recovered). (Found: C, 61.5; H, 7.5; N, 5.5. d_{25}^{25} , 1.0588. $C_{13}H_{13}O_4$ requires C, 61.7; H, 7.5; N, 5.5 per cent).

Hydrolysis of Ethyl α -Cyano- β -isopropylglutaconate : Formation of 2:6-Dihydroxy-4-isopropylpyridine (V).—In a typical experiment, the ester (2 g.) was mixed with concentrated hydrochloric acid (6 c.c.) and heated under reflux on a sand-bath. After 4 hours, hydrochloric acid (4 c.c.) was added

and the heating continued for another 4 hours. The product was then evaporated to one-third its bulk, when the hydrochloride of 2:6-dihydroxy-4-isopropylpyridine separated (1.1 g.). This was filtered, the hydrochloride suspended in water, made first alkaline with ammonia and then the solution acidified with acetic acid when the free base was deposited. It recrystallised from absolute alcohol in colourless rectangular prisms, m.p. $213-14^{\circ}$ (Gibson and Simonsen, *loc. cit.* give m.p. $213-14^{\circ}$). In its properties, it entirely agreed with those described by Gibson and Simonsen. It gives a purple colouration with alcoholic ferric chloride. An aqueous solution rapidly turns green in air, whilst an ammoniacal solution becomes blue. (Found: N, 9.7. $C_8H_{11}O_2N$ requires N, 9.2 per cent).

trans-β-isoPropylglutaconic Acid (I).—The mother-liquor from the above experiment after the filtration of the hydrochloride, was further concentrated, and then repeatedly extracted with ether. The ethereal solution was dried over magnesium sulphate and ether removed, when crude *trans-β-iso*propylglutaconic acid solidified (0.1 g.). Repeated crystallisations from a mixture of petroleum ether and acetone gave rectangular plates, m.p. 142° . It is easily soluble in water, alcohol, acetone and sparingly soluble in chloroform and benzene. [Found: C, 55.89, 55.77; H, 7.0, 7.12. Equiv., 85.5, 86.6. $C_8H_{12}O_6$ (dibasic) requires C, 55.82; H, 7.39 per cent. Equiv., 86.]

Conversion of the Above Acid into its Hydroxy Anhydride.—The acid (0.1 g.) was mixed with acetyl chloride (0.5 c.c.) and the mixture warmed on the water-bath for 15 minutes. After the removal of the excess of acetyl chloride in *vacuo* the anhydride remained as an oil which did not solidify. It gives an intense purple colouration with alcoholic ferric chloride which disappears on warming.

On mixing benzene solutions of the anhydride and *p*-toluidine, the semi-*p*-toluidide separated in prismatic needles, m.p. 150° .

Conversion of the Anhydride into the cis-Acid.—The anhydride (0.1 g.) was mixed with aqueous caustic potash (30 % 1.5 c.c.) and warmed on the water-bath at 35° for 3 hours. The product was acidified with hydrochloric acid, extracted with ether (4 times), the ethereal solution dried and ether removed, when the residue solidified. It was recrystallised from acetone, m.p. $129.5-130.5^{\circ}$. [Found: Equiv., 85. $C_8H_{12}O_6$ (dibasic) requires Equiv., 86].

The author's thanks are due to Prof. P. C. Guha for his interest in the work and for the kind encouragement he has given.

THE ACTION OF INORGANIC COLLOIDS ON ELECTRODEPOSITION OF NICKEL.

BY V. S. PURI AND V. S. BHATIA.

The electrodeposition of nickel sulphate bath on copper plates in the presence of Prussian blue, silver, ferric oxide and arsenious sulphide sols has been studied. A dull and very rough deposit is obtained in the presence of arsenious sulphide, while the presence of other sols yields a comparatively lustrous and smooth deposit. The nature and concentration of the sol seem to affect the nature and amount of deposit.

When a strip of zinc is dipped in a solution of lead acetate, a bright crystalline 'tree' is obtained. If glue has been added to the bath solution, an amorphous looking deposit results. A tin tree exhibits a similar phenomenon.

Mueller Bahntje (*Z. Electrochem.*, 1906, **12**, 317) found that copper deposited in the presence of colloids weighed 0.2% more than the metal deposited without the colloid. They found that gelatine had the most powerful effect, egg-albumin considerably less and gum and starch had no action. Investigators in the realm of electrodeposition of nickel have mainly occupied themselves in determining the influence of organic colloids on the nature of deposit. They have been using the above mentioned colloids and in certain cases, essential oils (*cf.* Mathers and Cockrum, *Met. Chem. Eng.*, 1914, **12**, 714; Mathers and Leible, *Chem. Abst.*, 1917, **11**, 1792). The investigation of the action of inorganic colloids forms the subject of the present paper.

EXPERIMENTAL.

The bath used was obtained by mixing equal volumes of nickel sulphate solution (78.56 g. per litre) and boric acid and potassium chloride (30 g. and 19 g. per litre respectively). Nickel sulphate was obtained by recrystallisation of the B.D.H. sample. Its concentration was determined by precipitation as barium sulphate in an aliquot portion. The other reagents were analytically pure.

Copper plates (6" x 1") were taken and rubbed till free from the oxide layer. They were rinsed with water and alcohol and dried before use.

The cathode was further subjected to the following cleansing treatment successively :

(a) It was dipped to form the cathode in a 5% solution of sodium hydroxide and sodium carbonate, and then rinsed with water.

(b) It was dipped in a 10% solution of KCN which was followed by a water rinse.

(c) It was electrically cleaned as in (a) and finally it was dipped in 10% sulphuric acid and washed with water and alcohol.

The loss in weight sustained by the cathode, as determined in five experiments, was approximately 0.004-0.005% of the weight of the plate. Current was adjusted till a uniform deposit of copper and nickel was obtained. Current of 0.2 ampere if passed for about 30 minutes gave a suitable deposit. An increase in the current strength resulted in the separation of nickel in a spongy form.

Three different colloids were tried. The addition of colloids produces no effect on the calculated theoretical amount of nickel deposited. The results are given below.

Prussian Blue Hydrosol.

M/28-Potassium ferrocyanide (20 c.c.) was taken and 60 c. c. of water were added to it. Glycerine (5 drops) was then mixed with it to stabilise the colloid. Ferric chloride (20 c. c., M/27-approximately) was then added drop by drop followed by continuous shaking. A deep blue coloration resulted.

Quantitatively there is no appreciable effect on the amount of metal deposited, but the nature of deposit undergoes a change as seen from the plates.

Plate B shows the nature of the surface when no colloid has been added, while Plate A exhibits the initial character of the cathode. It will be seen that the abrasion marks are straight and do not show any tendency to cross-grain. In the nickel-plated surface the pitch of the lines is definitely altered. The deposit is not very hard or coherent.

Plate C-side opposite the anode. Addition of 5 c. c. of the hydrosol resulted in the decrease in the number of abrasion marks and the size of the grain of the depositing nickel was much reduced. The plate after deposit took on a lustre which was absent in Plate B, and the deposit was hard.

With 10 c. c. of the hydrosol, the plate D has a marked resemblance to Plates A and B showing that the thickness of the deposit is just enough to cover the copper cathode, while it is insufficient to overlap the inherent defects of the plate.

With 15 c.c. of the hydrosol Plate E shows the effect of such an addition. A study of this leads one to conclude that the strength of the colloid has exceeded the upper limit of its optimum concentration. A close examination reveals that nickel has been loosely deposited. It appears that the large amount of the colloid renders considerable reduction in the size of the grain.

Silver Hydrosol.

The hydrosol was obtained by mixing 5 c.c. of 1 % silver nitrate solution with water so as to make 100 c.c. It was then warmed and to this 3 c.c. of 1% sodium carbonate solution were added. Freshly prepared tannin (1%, 1 to 3 c.c.) was dropped in gradually until a deep yellowish brown colouration developed. The solution became transparent on dilution.

Plate G shows the nature of the deposit when 5 c.c. of the above sol are added. It is noticed that the solution is more potent than the previous one. A definite reduction in grain size takes place all over the surface, and the cross-graining is also uniform. Plate H shows the effect of 15 c.c. of the sol. Under the magnification used, the surface appears to be almost without the abrasion marks. Such a plate can take a high polish subsequently with a marked ease. Besides, the deposit is hard and coherent.

Arsenious Sulphide Sol.

The sol was prepared in the usual manner by passing hydrogen sulphide through a saturated and filtered solution of arsenious oxide. Before boiling off the gas it was seen that all the arsenic had been precipitated as the sulphide. Hydrogen sulphide was removed by boiling and bubbling hydrogen gas through the colloidal solution.

The solution was mechanically stirred throughout the experiment otherwise the colloid settled down and altered the concentration of the sol, in the different parts of the bath.

It appears that the colloid hinders the deposition of the metal. There is a wide discrepancy between the theoretical and the observed values. Moreover, it was seen that the metal was deposited as a loose, black spongy mass and this was easily removed with water. Plate J shows the condition of such a plate when 5 c.c. of the colloid were added. Grain-size reduction did take place but the deposit was neither hard nor coherent. Plate K shows the nature of the surface at the bottom of the plate. As the concentration of the colloid was greater in the

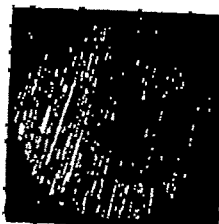
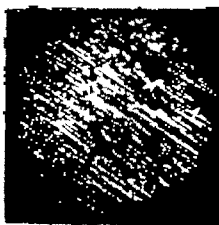
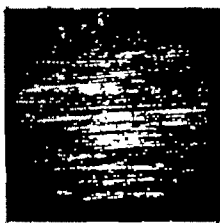
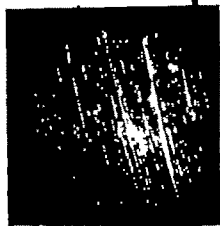
lower regions, small crystals of nickel could be perceptible. Even the naked eye can make out the roughness of the deposit which is dark and lacks lustre. In some places the copper is visible. This is shown in curved lines in Plate L.

DISCUSSION.

Grube and Ruess (*Z. Electrochem.*, 1921, 27, 45) showed that irrespective of the charge on the colloid, the nickel ions form a complex with the colloid, which travels towards the cathode due to cataphoresis. This complex is decomposed on coming into contact with the plate and the metal is deposited. The nature of deposit depends on the nature of the complex. This is corroborated by the fact that some arsenious sulphide is found embedded in the cathode. This may also lead us to favour the adhesion theory which assumes the formation of a colloidal wall, of about molecular thickness round the cathode. Nickel ions have to penetrate this wall before they can get deposited. Thus it may be likened to percolation. The time taken for the process may be considerable and it is plausible to presume that some of the depositing Ni ions are embedded in the wall and have had no time to get deposited, when the current is switched off. The deficiency in the deposit is thus explained by the nature of the wall. As_2S_3 has a negative effect because it has a tendency to flocculate and agglomerate. This does not give a uniformly thick wall. Prussian blue and silver hydrosols impart a lustre to the plate, since in these cases there is no flocculation and all the crystals are in one plane and thus maximum brilliance due to reflection is obtained.

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Received January 23, 1939



ENZYMES IN SNAKE VENOM. PART V. DETECTION OF DIPEPTIDASE, POLYPEPTIDASE, CARBOXY-POLYPEPTIDASE AND ESTERASE IN DIFFERENT SNAKE VENOMS.

BY B. N. GHOSH, P. K. DUTT AND D. K. CHOWDHURY.

In this paper the results so far obtained regarding the presence of cholin-esterase, and various peptidases in the venoms of a number of snakes, have been recorded.

In a series of papers (Ghosh *et al.*, *J. Indian Chem. Soc.*, 1936, **13**, 450, 627 ; 1937, **14**, 564 ; 1938, **15**, 471) it has been shown by us that the proteolytic enzyme present in snake venom resembles trypsin in all the properties investigated so far and hence suggestion has been made that the proteinase is identical with trypsin. A number of other enzymes in snake venom has also been detected recently. Roy (*Indian J. Med. Res.*, 1938, **26**, 241) has found that the venom of cobra (*Naja naja*) contains an esterase which hydrolyses ethyl butyrate. Iyengar and co-workers (*Current Sci.*, 1938, **7**, 53) and also Ghosh and co-workers have found that cobra venom contains an enzyme which can hydrolyse acetylcholine. In addition to these, we have detected the presence of such enzymes as polypeptidase, dipeptidase and carboxypolypeptidase in the venoms of snakes belonging both to the *Colubridae* and the *Viperidae* groups. The results so far obtained regarding the presence of polypeptidase, dipeptidase, carboxypolypeptidase and choline-esterase in different venoms are recorded in this paper.

Dipeptidase.

The properties of purified dipeptidase, prepared from yeast and gut of animals, have been studied by Grassman and Dyckerhoff (*Z. physiol. Chem.*, 1928, **179**, 41) and Waldschmidt-Leitz, Balls and Waldschmidt-Grassser (*Z. physiol. Chem.*, 1926, **166**, 68). They have found that this enzyme can hydrolyse dipeptides like glycylglycine which contains no asymmetric carbon atom and hence optically inactive and glycyl-L-leucine which is optically active, but it cannot hydrolyse dipeptides like glycyl-D-leucine, which does not occur in nature. In the course of our investigation on the enzymes in snake venoms, we have found a dipeptidase in the venoms of cobra (*Naja*

naja), banded krait (*B. Fasciatus*), *Echis carinata* and *Vipera russellii*. Like the gut or yeast dipeptidase, this peptidase in venom can hydrolyse glycylglycine and *l*-leucylglycine, but not the *dextro*-rotatory forms of the dipeptides like *d*-leucylglycine. This will be evident from the data recorded in Table I. The course of hydrolysis was followed by Sørensen's method of formol titration. The experimental procedure was exactly the same as described by Ghosh and co-workers (*loc. cit.*). The concentration of the substrates used was 0.008*M* and the concentration of the venoms-used was 0.1 % in each case. The p_H of the substrate-venom solution was 7.0.

TABLE I.

0.01*N*-NaOH required (in c.c.) for titration of 5 c.c. of the solution after 24 hours' incubation at 36°.

Cobra venom.				
Substrate.		Control.	Venom+substrate.	Diff
<i>l</i> -Leucyl- <i>l</i> -tyrosine	...	1'52 c.c.	2'42 c.c.	0'90 c.c.
<i>l</i> -Leucylglycine	...	1'30	1'74	0'44
<i>d</i> -Leucylglycine	...	1'30	1'30	0'00
Glycylglycine	...	8'80	9'45	0'65
Russell's viper.				
<i>l</i> -Leucyl- <i>l</i> -tyrosine	...	1'55 c.c.	2'65 c.c.	1'10 c.c.
<i>l</i> -Leucylglycine	...	1'25	1'60	0'35
<i>d</i> -Leucylglycine	...	1'25	1'25	0'00
Glycylglycine	...	8'80	9'55	0'75
B. Fasciatus.				
<i>l</i> -Leucyl- <i>l</i> -tyrosine	...	1'55 c.c.	2'11 c.c.	0'56 c.c.
<i>l</i> -Leucylglycine	...	1'20	1'85	0'65
<i>d</i> -Leucylglycine	...	1'25	1'25	0'00
Glycylglycine	...	8'70	9'40	0'70

TABLE I (contd.).

Echis carinata.				
L-Leucyl-L-tyrosine	...	1'60 c.c.	4'80 c.c.	3'20 c.c.
L-Leucylglycine	...	1'40	2'55	1'15
D-Leucylglycine	..	1'40	1'40	0'00
Glycylglycine	...	8'75	9'55	0'80

Polypeptidase.

The properties of purified polypeptidase obtained from yeast and gut of animals have been investigated by Grassman and Dyckerhoff (*loc. cit.*) and Waldschmidt-Leitz and Balls (*Ber.*, 1931, 68, 1203). It has been found that this enzyme attacks the naturally occurring *laevo*-rotatory forms of the polypeptides only and not the *dextro*-rotatory forms. We have found the presence of this enzyme in the venoms of *Naja naja*, *Vipera russellii*, *Echis carinata* and *B. Fasciatus*. The results are recorded in Table II. The concentration of the solutions with respect to the substrates was 0'008M and with respect to the venoms was 0'1 % in all the cases recorded below. It will be noticed from the data in Table II that the enzyme can hydrolyse L-leucylglycylglycine and not D-leucylglycylglycine. This seems to indicate that the polypeptide molecule is attacked by the enzyme on the end containing the free amino group.

TABLE II.

0'01N-NaOH solution required (in c.c.) for titration of 5 c.c. of the solution after 24 hours' incubation at 36°.

Cobra venom.				
Substrate.	Control.		Venom + substrate.	Diff.
<i>L</i> -Leucylglycylglycine ...	1'40 c.c.		5'40 c.c.	4'00 c.c.
<i>D</i> -Leucylglycylglycine ...	1'40		1'40	0'00
Russell's viper.				
<i>L</i> -Leucylglycylglycine ...	1'35 c.c.		2'36 c.c.	1'01 c.c.
<i>D</i> -Leucylglycylglycine ...	1'35		1'35	0'00

TABLE II (contd.).

B. Fasciatus.			
L-Leucylglycylglycine ...	1'40 c.c.	4'10 c.c.	2'70 c.c.
D-Leucylglycylglycine ...	1'40	1'40	0'00
Echis carinata.			
L-Leucylglycylglycine ...	1'40 c.c.	5'66 c.c.	4'26 c.c.
D-Leucylglycylglycine ...	1'40	1'40	0'00

Carboxypolypeptidase.

Carboxypolypeptidase has been obtained in crystalline form by Anson (*Science*, 1935, **81**, 461). Bergmann, Zervas and Schleich (*Z. physiol. Chem.*, 1934, **224**, 45) have shown that it can hydrolyse peptides of the form $\text{XCONH}\cdot\text{CRH}\cdot\text{COOH}$, where X and R stand for univalent radicals. We searched for this enzyme in the venoms of *Naja naja*, *B. fasciatus*, *Echis carinata* and *Vipera russellii* using chloroacetyltyrosine as substrate. It will be noticed from the data recorded in Table III that the venoms mentioned above contain appreciable amount of carboxypolypeptidase. In the following experiments the concentration of the different venoms in the solutions was 0'1%, the concentration of the substrate was 0'008M, and the p_H of the solution 7'0 in each case.

TABLE III.

Substrate=Chloroacetyl-L-tyrosine.

0'01N-NaOH solution required (in c.c.) for the titration of 5 c.c. of the solution after 24 hours' incubation at 36°.

Type of venom used.	Control.	Venom + substrate.	Diff.
<i>Naja naja</i> ...	2'70	3'46	0'76
<i>Vipera russellii</i> ...	2'65	3'20	0'55
<i>B. fasciatus</i> ...	2'65	3'20	0'55
<i>Echis carinata</i> ...	2'70	5'25	2'55

Choline-esterase.

The existence of a choline-esterase was first suggested by Dale (*J. Pharm. Expt. Therap.*, 1914-15, 6, 141). It occurs in the blood and tissues such as heart muscle, intestinal mucosa, etc. of animals. It has been shown by Stedman and co-workers (*Biochem. J.*, 1932, 26, 2056; 1933, 27, 1055) and Plattner and co-workers (*Pfl. Arch.*, 1930, 226, 19) that it is specific in its action since it does not parallel the lipase content of various organs. Iyengar and co-workers (*loc. cit.*) have recently found that Cobra (*Naja naja*) venom possesses considerable cholin-esterase activity, whereas *Vipera russellii* shows practically no such activity. We have also found that *Naja naja* and *B. Fasciatus* venoms possess marked cholin-esterase activity, while the venoms of *Echis carinata*, *Vipera russellii* and *Crotalus-t-terrificus* show no such activity. This will be noticed from the data recorded in Table IV. Since acetylcholine is liberated from nerve-endings and mediates in the transmission of nerve impulse and since in cobra poisoning there occurs paralysis of the nervous system, Iyengar and co-workers (*loc. cit.*) put forward the hypothesis that the neurotoxin of cobra venom is probably identical with choline-esterase. They meant thereby that when a cobra neurotoxin is introduced into the animal system in sufficient amount, it causes immediate destruction of the acetylcholine liberated, and thus produces paralysis of the nervous system. We have, however, found that the purified cobra neurotoxin (M.L.D 1/90 mg.) prepared by us does not possess choline-esterase activity. Furthermore when solutions of crude cobra and *B. Fasciatus* venoms are heated at 60° or 70°, they completely lose their choline-esterase activity, while their neurotoxic activity remains practically unaltered. This will be evident from the data recorded in Table V. In these experiments the concentration of the substrate solutions was 0.625% in those cases where cobra venom was used; in the other cases it was 0.315%. The p_H of the solutions was adjusted to 7.0.

TABLE IV.

0.0143N-NaOH required (in c. c.) for the titration of 10 c. c. of the solution after 3 hours' incubation at 36°.

Type of venom.	Conc. of venom.	Control.	Venom + substrate.	Diff.
naja Naja	0.0125%	4.05	10.90 c.c.	6.25 c.c.
B. Fasciatus	0.0250	4.10	15.80	11.75
Vipera russellii	0.1250	4.10	4.10	0.00
Echis carinata	0.0125	4.10	4.10	0.00
Crotalus-t-terrificus	0.0125	4.10	4.10	0.00

TABLE V.

0.0143N NaOH required (in c.c.) for the titration of 10 c.c. of the solution after 3 hours' incubation at 36°.

Type of venom.	Venom heated.	Conc. of venom.	Control	Venom + substrate.	Diff.	Toxicity remain. ing.
Naja naja crude	60°	0.0125%	4.05 c.c.	4.05 c.c.	0.00 c.c.	>95%
	70°	0.0125	4.05	4.05	0.00	>90
Naja naja purified neurotoxin	36°	0.0125	4.05	4.05	0.00	100
B. Fasciatus	60°	0.025	4.05	4.10	0.05	>95
	70°	0.025	4.05	4.05	0.00	>80

This inquiry was conducted with the help of a grant from the Indian Research Fund Association to whom our thanks are due. We would also thank Prof. K. H. Slotta of the Institute Butantan for kindly supplying the *crotalus-t-terrificus* venom.

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Received January 20, 1939.

ON THEORIES OF ADSORPTION INDICATORS.

BY S. G. CHAUDHURY AND M. K. INDRA.

The cataphoretic velocities of silver halides with or without the dye indicators under the exact conditions of titrations have been measured. These observations are not in good agreement with the theory of adsorption indicators as propounded by Fajans or Kolthoff.

The applications of acidic and basic dyes as indicators to argentometric and other similar volumetric titrations have come into prominence mainly through the pioneering work of Fajans, Kolthoff and their collaborators.*

These titrations are based on the assumption that the indicators used change colour just when one or other of the constituent ions of the precipitate is in very slight excess.

When silver nitrate is gradually added to potassium iodide in presence of eosin as indicator, negatively charged colloidal silver iodide is formed in excess of iodide ions, and so cannot adsorb the negative eosinate ions. Thus the greenish fluorescence of the indicator ion persists till the end point is reached where the colloid usually coagulates. As soon as a slight excess of silver nitrate over that corresponding to the equivalent amount is added, the precipitate becomes positively charged and adsorbs negative eosinate ions and the colour of the precipitate changes suddenly and fluorescence disappears.

In reverse titration, the red colour, first formed, due to the adsorption of eosin ions by positively charged silver halide sol vanishes just when a slight excess of the halide is added and fluorescence again reappears due to the liberation of the secondarily adsorbed eosin ions by negatively charged chlorine ions (*cf.* Fajans).

Kolthoff (*Chem. Rev.*, 1935, 16, 87) points out that the theory suggested above is not adequate, because the disappearance of fluorescence and the slight appearance of the colour of the precipitate takes place a little before the equivalent point is reached, where the precipitate is assumed to be negatively charged, and therefore electrical adsorption of the dye ions is not possible. Kolthoff suggests that the colour change arises out of the exchange by adsorption of the negatively charged halide ions of the surface with the fluorescent dye ions in solution.

There is, however, no experimental evidence regarding the manner of variation of the charge of these precipitates (either in the colloidal state or

* Fajans, "Radio-elements and Isotopes", 1931, p. 96 ; Fajans and Ardey Grüz., *Z. physikal. Chem.* 1931, 188A, 97 ; Kolthoff *et. al.*, *Chem. Rev.*, 1935, 16, 87.

when thrown down) under the conditions of titration to support assumptions made in the theories mentioned above. Fajans' theory was based on the observation of Lottermoser (*J. pr. Chem.*, 1905, **12**, 39; 1905, **13**, 374; Lottermoser and Roths, *Z. physikal. Chem.*, 1908, **62**, 359) on the variation of the charge of silver halides in excess of either silver or halide ions. It seemed desirable, therefore, to measure the charge of the silver halides under the actual conditions of titrations. The present paper furnishes only experimental facts.

EXPERIMENTAL.

The cataphoretic velocities of the silver halide particles either in the precipitated or in the colloidal state have been measured by the micro-cataphoretic method described before (Mukherjee, Chaudhury and Bhabak, *J. Indian Chem. Soc.*, 1930, **13**, 370; Mukherjee, Chaudhury and Sen-Gupta, *ibid.*, 1936, **13**, 421; Chaudhury and Sen-Gupta, *ibid.*, 1936, **13**, 670).

The velocities were observed at a single height (at 0.21 depth of the cell, where there is no endosmosis). It has been verified experimentally that the velocity observed at this height agrees well with that calculated from the observed velocities at half and one-sixth height.

TABLE I.

0.1N-KI (0.5 c.c.) and 0.1N-AgNO₃ (2 c.c.) used. Total volume = 100 c.c. containing 0.3 mg. of sodium eosinate.

Direct		Reversed		Mean		$V \times 10^5$.
Time.	Current.	Time.	Current	Time.	Current.	
13.6 sec.	0.3.8 m amp.	12.0 sec.	0.352 m.amp.			
13.2	0.308	10.0	0.374	11.85 sec.	0.347 m. amp.	12.5
13.4	0.308	10.0	0.396			
13.1	0.33	9.5	0.396			
Sp. conductivity. = 2.711×10^{-4} mho.						
13.0	0.33	11.5	0.352			
13.2	0.33	11.2	0.352	11.96	0.348	12.41
12.8	0.33	10.8	0.374			
12.6	0.341	10.6	0.374			

Sp. conductivity. = 2.715×10^{-4} mho

Experiments were carried out with silver nitrate and potassium chloride, potassium bromide, and potassium iodide with eosin, fluorescein and methyl violet as indicators.

RESULTS AND DISCUSSIONS.

I. Considerations of Experimental Results from the point of view of Fajans and Kolthoff when Lattice Ions of the Same sign as that of the Indicator Ions are in Excess.

The experimental results given in Table II are not in good agreement with either the theory of Fajans (*loc. cit.*) or that of Kolthoff (*loc. cit.*).

TABLE II.

Total volume = 100 c.c.

Halide (0.1N).	0.1N-AgNO ₃ .	Dye.	Nature of charge.	$V \times 10^5$.
KI (4 c.c.)	3.85 c.c.	Without dye	-ve	35.1
	3.85	Eosinate (0.3 mg.)	-ve	35.04
	3.85	Fluoresceinate (0.3 mg.)	-ve	35.5
KBr (4 c.c.)	3.85	Without dye	-ve	34.04
	3.85	Fluoresceinate (0.5 mg.)	-ve	34.85
	3.85	Eosinate (0.3 mg.)	-ve	34.46
KCl (4 c.c.)	3.85	Without dye	-ve	31.2
	3.85	Fluoresceinate (0.3 mg.)	-ve	31.38
	3.85	Eosinate (0.3 mg.)	-ve	36.1

0.1N-Silver nitrate (3.85 c.c.) were added to 4 c.c. of a 0.1N-potassium halide (KCl, KBr and KI) with or without 0.3 mg. of sodium eosinate or fluorescein. With or without the indicator, the values of c. v. and hence the corresponding densities of charge of the colloidal Ag-halide are negative and the same in magnitude. According to Fajans, there should be no electrical adsorption of the dye ions under these conditions. These facts therefore appear to support the postulations of Fajans (*loc. cit.*). There are, however, exceptions. With eosin and silver chloride, an increase in c.v. is observed in presence of the dye ion. With the basic indicator used, it has been found that adsorption of the positive dye ion takes place even when silver ions are in excess, thus supporting the similar observations of Hodakow (*Z. physikal. Chem.*, 1927, 127, 43; 1927, 129, 118) obtained analytically. Also at this stage of titration, there is absolutely no fluorescence* in the case of fluorescent indicators and a distinct reddish colour is to be

* The disappearance of fluorescence may be partly attributed to the quenching effect produced by the increasing concentrations of hydrogen ions and potassium nitrate formed as a result of the reaction

observed in the precipitate. These facts are not in agreement with the theory of Fajans as already pointed out by Kolthoff (*loc. cit.*). In order to account for this change of colour, Kolthoff (*loc. cit.*) assumed a sort of exchange adsorption between ions of the same sign on the surface and the dye ions in the solution. Thus according to Kolthoff there ought to be a change in the c. v. of the silver halides in presence of indicator solutions, for the reasons stated below.

(i) The dye ion is divalent* and a displacement of one halide ion by one dye anion (*cf.* as assumed by Kolthoff, *loc. cit.*) should have increased the negative charge. We find, however, that the c. v., *i.e.*, the density of charge, is not altered. In order to explain this constancy of charge from the point of view of Kolthoff one has to assume the displacement of exactly two chloride ions from the surface, which is rather unlikely.

(ii) Even if two halide ions are replaced by one indicator ion (which is, however, not probable), the surface character of the colloid complex and hence its power of adsorption, may change.

II. Considerations of the Theory of Fajans and Kolthoff, when there is an Excess of Lattice Ions of Opposite Sign to that of the Indicator Ions.

The c. v. of the colloidal halides, either in excess of silver or halide ions, with and without the dye, have been determined and are given in Tables III, IV, Va, Vb.

TABLE III.

Silver iodide.

0.1N-KI and 0.1N-AgNO₃ taken in a total volume of 100 c.c.

0.1N-KI.	0.1N-AgNO ₃ .	Without dye.		With 0.3 mg. of Na-eosinate		With 0.3 mg. of Na-fluoresceinate	
		Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.
1.5 c.c.	2.0 c.c.	6-9 min.	22.3	6-9 min.	14.8	4-7 min.	13.65
		14-16	22.47	20-24	15.7	12-15	14.55
		22-25	22.75	30-32	15.46	21-25	15.6
							16.42
				3 hrs.	charge -ve	3 hrs.	charge -ve
1.9	2.0	8-12	18.3	4-7 min.	-13.12	5-9 min.	-13.95
		19-21	20.3	12-15	-16.86	14-17	-23.1
		28-31	20.4	20-23	-15.93	23-27	-21.8
				31-35	-14.15		

* The p_H values of all the halide solutions and silver nitrate lie between 6 and 7 except that of KI which is a little alkaline. During precipitation, the p_H value is lowered in a number of cases but never comes down below 5 (*cf.* D. Mukherjee, *J. Indian Chem. Soc.*, 1935, 12, 748). Under these conditions, the indicator anions are divalent.

TABLE IV.

*Silver iodide.*0.1N-KBr and 0.1N-AgNO₃ taken. Total volume = 100 c.c.

0.1N-KBr. 0.1N-AgNO ₃ .		Without dye.		With 0.3 mg. of Na-eosinate.		With 0.3 mg. of Na-fluoresceinate.	
		Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.
1.7 c. c.	2.0 c. c.			5-8 min.	9.55		
				11-14	10.75		
				17-20	10.34		
				24-28	9.34		
				31-34	8.67		
				38-44	8.76		
				3 hrs.	-15.89		
1.9	2.0	8-11 min.	12.85	7-10 min.	8.71	5-9 min.	-23.18
		18-22	17.33	13-15	9.14	17-21	-27.08
		28-32	16.17	20-24	7.18		
				27-30	5.93		
				3 hrs.	-19.37		

TABLE V(a).

*Silver iodide.*0.1N-KCl and 0.1N-AgNO₃ taken. Total volume = 100 c. c.

0.1N-KCl. 0.1N-AgNO ₃ .		Without dye.		With 0.3 mg. of Na-fluoresceinate.	
		Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.
1.5 c. c.	2.0 c. c.	6-9 min.	21.43	8-12 min.	-5.55*
		14-18	25.17	3 hrs.	-13.57
				All -ve	
1.9	2.0	5-9	9.09		
		15-18	12.2	6-10 min.	-8.2
2.0	1.0	5-9	-30.75		
2.0	1.5	6-10	-27.38		
2.0	1.9	6-9	-22.24		
		15-19	-21.3		

* Immediately after filling the cell, particles of both sign were visible moving slowly in these cases. The number of negatively charged particles appeared to be greater.

TABLE V(b).
Silver Iodide.

0.1N-KCl and 0.1N-AgNO ₃ taken.		Total volume = 100 c.c.			
0.1N-KCl.	0.1N-AgNO ₃ .	With 0.3 mg. methyl violet.		Without dye.	
		Time after mixing.	$V \times 10^5$.	Time after mixing.	$V \times 10^5$.
1.5 c. c.	2.0 c. c.	6-10 min.	31.4	6-9 min.	21.43
				14-18	25.17
1.9	2.0	5-9	19.83	5-9	9.09
		14-18	20.87	15-18	12.2
2.0	1.5	4-8	18.8	6-10	-27.38
		13-15	21.5		
2.0	1.9	5-9	10.74	6-9	-22.24
		15-18	12.64	15-19	-21.3

It would be found that in the majority of cases, increasing quantities of potassium halide was added to a constant quantity of silver nitrate (0.1N; 2 c. c. in 100 c. c.) with or without the indicator (0.3 mg.) keeping the concentration of silver nitrate in excess. As expected, the charge is always positive in absence of the dye. In presence of the indicators, the charge is also mostly positive. The value of the c. v. with dye is usually lower than that without the dye. The positive value of c. v. in presence of the dye diminishes with time and finally becomes negative in some cases. When, however, the amount of potassium iodide (0.1N) added is 1.9 c. c., the charge is negative from the very beginning though without dye, the charge is positive. It appears that under these conditions, the type of electrical secondary adsorption and the subsequent deformation of the dye ions, as visualised by Fajans (*loc. cit.*), takes place. But the following facts which do not agree with Fajans' theory should also be kept in view

(i) The change of colour is instantaneous and does not vary with time whereas the charge varies with time.

(ii) This colour change is to be noticed from the very beginning of titration, that is to say when 2 to 3 c. c. of potassium iodide have been added to 2 c. c. of silver nitrate.

With 1.9 c. c. of KBr (Table IV), the charge is positive at the beginning in presence of sodium eosinate, but finally becomes negative. On the other hand with sodium fluoresceinate and KCl (*cf.* Table Va), the charge is always negative. Sometimes both the positively and negatively charged particles were visible immediately after mixing.

With methyl violet, a basic indicator with a positively charged dye cation (and KCl, Table Va), the charge has been found to be always positive irrespective of whether silver ions or chlorine ions are present in excess

(cf. the work of Hodakow, *loc. cit.*). Sometimes particles of both sign were visible just after mixing. These facts indicate the specific nature of the effects-observed for particular pairs of adsorbents and indicators.

III. Consideration of the Fajans-Kolthoff Theory when the Precipitate is formed from Equivalent Quantities.

When equivalent quantities of silver nitrate and potassium halides are mixed, particles of both sign are persistently visible, when the dye is absent. In presence of the acidic dye ions the charge is found to be always negative (cf. Tables VI, VII, VIII) and the precipitate coloured.

TABLE VI.

KI and AgNO_3 in equivalent quantities. Total volume = 100 c.c.

0.1N-KI	0.1N- AgNO_3	Nature of the charge.	Time after mixing.	$V \times 10^5$.
2 c.c. Without dye	2 c.c.	Both +ve and -ve.	3 hrs.	27.0
2 Without dye	2	After 3 hrs. all -ve.	3 hrs.	26.57
2 0.3 mg. Na salt of eosin	2	Both +ve and -ve.	5-9 min.	23.43
		After 3 hrs. all -ve.		
2 0.3 mg. Na salt of eosin	2	-ve	15-18 5-9	26.65 22.06
2 0.3 mg. Na salt of fluorescein	2	-ve	18-20 5-9 16-20	25.12 24.65 31.9
1 0.3 mg. Na salt of fluorescein.	2	-ve	4-7 15-19	24.83 30.15

TABLE VII.

KBr and AgNO_3 in equivalent quantities. Total volume = 100 c.c.

0.1N-KBr.	0.1N- AgNO_3	Dye.	Nature of charge.	Time after mixing.	$V \times 10^5$.
2 c.c.	2 c.c.	...	Both +ve and -ve.	60 min.	16.35
2	2	...	After 1 hr. all -ve.	60	16.4
2	2	0.3 mg. Na-fluoresceinate.	Both +ve and -ve.	7-9	29.62
			After 1 hr. all -ve.		
2	2	0.3 mg. Na-fluoresceinate.	-ve	15-19 6-9	32.45 29.3
2	2	0.3 mg Na-eosinate	-ve	15-18 7-10	31.8 17.5
2	2	0.3 mg Na-eosinate	-ve	15-18 6-9 15-19	18.72 16.88 18.6

TABLE VIII.

KCl and AgNO₃ in equivalent quantities. Total volume = 100 c.c.

0.1N-KCl.	0.1N-AgNO ₃ .	Nature of the charge.	Time after mixing.	$V \times 10^5$.
2 c.c. Without dye	2 c.c.	Both +ve and -ve.		
		After 1 hr. all -ve.	1 hr.	22.66
2 Without dye	2	Both +ve and -ve.		
		After 1 hr. all -ve.	1	21.92
2 0.3 mg. Na salt of fluorescein.	2	-ve		8.6
			1	22.4
2 0.3 mg. Na salt of fluorescein	2	-ve		8.77
			1	15.65
2 0.3 mg. Na salt of eosin	2	-ve		28.56
			1	32.46
2 0.3 mg Na salt of eosin	2	-ve		24.5
			1	27.54

It is to be noted that the c.v. values of silver iodide and silver bromide are increased in presence of fluoresceinate ions, but do not change when eosinate ions are added (*cf.* Tables VI and VII). On the other hand, the c.v. values of silver chloride are increased in presence of eosinate but not in presence of fluoresceinate ions. Thus this clearly shows the specific nature of the adsorption of these ions. Moreover, we cannot assume the adsorption of either kind as the cause of the change of the colour of the precipitate, when the c.v. values are not altered (*cf.* discussion I; also *cf.* discussion II when the c.v. values are changed). Thus it is clear that the theories put forward either by Fajans (*loc. cit.*) or by Kolthoff (*loc. cit.*) are not in general agreement with all the facts observed.

Our best thanks are due to Prof. J. N. Mukherjee, D.Sc. for facilities given.

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Received January 19, 1939.

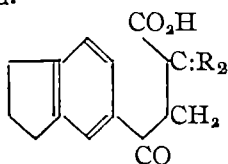
STUDIES IN DEHYDROGENATION. PART III.*

BY SURESH CHANDRA SEN-GUPTA.

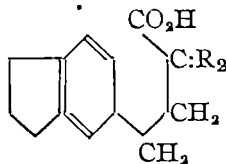
In the present communication the syntheses and selenium dehydrogenation of 6:7-cyclopentenotetrahydronaphthalene and 2:2-dimethyl-6:7-cyclopentenotetrahydronaphthalene have been described. The former has been dehydrogenated to 6:7-cyclopentenonaphthalene and the latter gives 6:7-cyclopenteno-2-methylnaphthalene

With the idea of studying the selenium dehydrogenation of hydroaromatic compounds containing a fused cyclopentane ring, 6:7-cyclopentenotetrahydronaphthalene and 2:2-dimethyl-6:7-cyclopentenotetrahydronaphthalene have been synthesised. It was found that during dehydrogenation with selenium at 300-340°, the cyclopentane ring was not affected in either case and the latter compound, which contained *gem*-dialkyl groups, a quaternary methyl group was easily eliminated when heated with selenium in a sealed tube at 320°. This result is in line with the observation of Ruzicka and Seidel (*Helv. Chim. Acta*, 1936, 19, 424) that α - and β -methylhydrindenes are unaffected when heated with selenium at 350-400° but are converted into naphthalene at 450°.

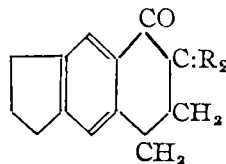
For the synthesis of 6:7-cyclopentenotetrahydronaphthalene, succinic anhydride was condensed with hydrindene in nitrobenzene solution in presence of anhydrous aluminium chloride when β -5-hydrindoylpropionic acid (I, R=H) was formed. The constitution of this keto-acid follows from its oxidation with alkaline permanganate to trimellitic acid. The keto-acid (I, R=H), on reduction with amalgamated zinc and hydrochloric acid, gives γ -5-hydrindylbutyric acid (II, R=H), which on cyclisation with 85% sulphuric acid gives 6:7-cyclopenteno-1-keto-1:2:3:4-tetrahydronaphthalene (III, R=H) in 65% yield. That the cyclisation proceeds in the manner indicated, with the formation of a linear product is proved by the oxidation of the cyclic ketone (III, R=H) to 1:2:4:5-benzenetetracarboxylic acid.



(I)



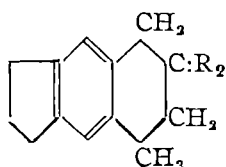
(II)



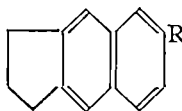
(III)

[*pr. Chem.*, 1938, 161, 82.

This cyclic ketone on reduction by the Clemmensen method gives 6 : 7 *cyclopenteno*-1 : 2 : 3 : 4-tetrahydronaphthalene (IV, R=H), which on dehydrogenation with selenium at 300-340° yields 6 : 7-*cyclopentenonaphthalene* or 5 : 6-benzohydrindene (V, R=H).



(IV)



(V)

In a similar way 2 : 2-dimethyl-6 : 7-*cyclopentenotetrahydronaphthalene* was synthesised, starting from *as*-dimethylsuccinic anhydride and hydrindene. The Friedel-Craft's reaction was carried out in nitrobenzene solution yielding mainly $\alpha\alpha$ -dimethyl- β -5-hydrindoylpropionic acid (I, R=Me). The position of the keto group in this acid is proved by its oxidation with alkaline permanganate to trimellitic acid. The $\alpha\alpha$ -positions of the methyl groups have been assumed from analogy with the other keto-acids, synthesised previously from *as*-dimethylsuccinic anhydride and aromatic hydrocarbons. On reduction with amalgamated zinc and hydrochloric acid, the keto-acid (I, R=Me) gives $\alpha\alpha$ -dimethyl- γ -5-hydrindylbutyric acid (II, R=Me), which on cyclisation with 85% sulphuric acid gives about 85% of the theoretical yield of 1-keto-6 : 7-*cyclopenteno*-2 : 2-dimethyl-1 : 2 : 3 : 4-tetrahydronaphthalene (III, R=Me). The structure of this linear cyclisation product is proved by its oxidation to 1 : 2 : 4 : 5-benzenetetracarboxylic acid. The cyclic ketone (III, R=Me) on reduction by Clemmensen's method gives 2 : 2-dimethyl-6 : 7-*cyclopenteno*-1 : 2 : 3 : 4-tetrahydronaphthalene (IV, R=Me). Selenium dehydrogenation of this substance was carried out in a sealed tube at 300-320° giving a good yield of 6 : 7-*cyclopenteno*-2-methylnaphthalene.

EXPERIMENTAL.

β -5-Hydrindoylpropionic Acid (I, R=H).—To a cold solution of aluminium chloride (32 g.) in nitrobenzene (150 c.c.) a mixture of hydrindene (23 g.) and succinic anhydride (20 g.) was slowly added. The mixture was kept in the ice-bath for 6 hours and at the room temperature

for 12 hours, when it was decomposed with ice and hydrochloric acid. Excess of nitrobenzene and hydrindene were removed in steam, the solid product dissolved in dilute sodium carbonate solution, the solution was filtered and acidified with hydrochloric acid. The separated acid was twice crystallised from glacial acetic acid and was obtained as cubes, m. p. 122-24°. This was finally crystallised from alcohol (charcoal) in colourless cubes, m. p. 123-24°, yield 18 g. (Found : C, 71.4 ; H, 6.4. $C_{13}H_{14}O_3$ requires C, 71.6 ; H, 6.4 per cent).

Oxidation of β -5-hydrindoylpropionic acid with alkaline potassium permanganate solution was carried by dissolving the acid (1 g.) in 100 c. c. of 5% caustic soda solution and heating with excess of permanganate solution on the steam-bath for 4 hours. The excess of permanganate was destroyed with the addition of a little alcohol and the alkaline solution filtered, acidified with hydrochloric acid and evaporated on the steam-bath. The residue was repeatedly extracted with ether and the solid residue was crystallised from concentrated hydrochloric acid (charcoal) in white granules, m. p. 214° with forthring, m. p. of trimellitic acid being 216°.

γ -5-Hydrindylbutyric Acid (II, R=H).—A mixture of β -5-hydrindoyl propionic acid (9 g.), amalgamated zinc (50 g.) and concentrated hydrochloric acid (50 c.c.) was allowed to stand for 3 hours at the ordinary temperature and then heated to boiling for 24 hours. The product was extracted with ether, the solvent distilled off and the oily residue extracted with sodium carbonate solution and filtered. The reduced acid separated out as an oil on acidifying the solution with hydrochloric acid, but readily solidified. This was collected and distilled, when it came over at 190-92°/6 mm. as a colourless oil which quickly solidified. It crystallised from petroleum ether (b.p. 40-60°) in long needles, m. p. 56°, yield 6.2 g. (Found : C, 76.3 ; H, 7.9. $C_{13}H_{16}O_2$ requires C, 76.5 ; H, 7.8 per cent).

6 : 7-cycloPenteno-1-keto-1 : 2 : 3 : 4-tetrahydronaphthalene (III, R=H).—A mixture of the preceding hydrindylbutyric acid (7.2 g.), concentrated sulphuric acid (21.6 c.c.) and water (7.2 c.c.) was heated on the steam-bath for 1½ hours with stirring. The product was poured on ice, extracted with ether, the ether extract washed with dilute ammonia and water, dried with potassium carbonate and ether distilled off. The residual liquid distilled at 167°/6 mm as a colourless oil, yield 4.5 g. (65% of theory) (Found : C, 83.8 ; H, 7.5. $C_{13}H_{14}O$ requires C, 83.9 ; H, 7.5 per cent).

Oxidation of 6 : 7-cycloPenteno-1-keto-1 : 2 : 3 : 4-tetrahydronaphthalene with Alkaline Permanganate.—The cyclic ketone (1 g.) was added to

200 c.c. of 5% caustic soda solution and boiled in a brine-bath for 12 hours with excess of potassium permanganate solution. After destroying the excess of permanganate with alcohol, the alkaline solution was filtered, acidified with hydrochloric acid and evaporated. The residue was extracted with ether and on distilling off the solvent a solid product was obtained which crystallised from concentrated hydrochloric acid in needles, m.p. 275-76° with previous shrinking. The acid seemed to have been partially converted into the anhydride, so it was heated with acetyl chloride for 2 hours and acetic acid and acetyl chloride removed under reduced pressure. The anhydride, thus produced, was kept in a desiccator over caustic potash, m.p. 284-85° (the di-anhydride of 1 : 2 : 4 : 5-benzenetetracarboxylic acid melts at 286°).

6 : 7-cycloPenteno-1 : 2 : 3 : 4-tetrahydronaphthalene (IV, R=H).—The foregoing ketone (4.8 g.) was reduced by heating with amalgamated zinc (25 g.) and hydrochloric acid (30 c.c.) for 24 hours. The product was extracted with ether, washed, dried, ether removed and the residue distilled over sodium as a thin colourless liquid, b.p. 125-26°/6 mm., yield 3.1 g. (Found : C, 90.8 ; H, 9.2. $C_{13}H_{18}$ requires C, 90.7 ; H, 9.3 per cent).

Selenium Dehydrogenation of 6 : 7-cycloPenteno-1 : 2 : 3 : 4-tetrahydronaphthalene to 5 : 6-Benzohydrindene.—The hydrocarbon (3 g.) was heated with powdered selenium (5 g.) at 300-310° for 8 hours and at 320-345° for 16 hours in a metal-bath. Part of the dehydrogenated product sublimed into the neck of the flask. The product was thoroughly extracted with ether, the solvent evaporated and the slightly coloured crystalline residue distilled over sodium under reduced pressure as a colourless solid crystallising from alcohol in flakes, m.p. 94°. (Found : C, 92.7 ; H, 7.2. $C_{13}H_{12}$ requires C, 92.2 ; H, 7.1 per cent). The *picrate* was prepared in alcoholic solution and it crystallised from alcohol in golden yellow needles, m.p. 120-21°. (Found : C, 57.3 ; H, 3.8. $C_{19}H_{15}O_7N_3$ requires C, 57.4 ; H, 3.8 per cent).

αα-Dimethyl-β-5-hydrindoylpropionic Acid (I, R=Me) was prepared from *αα*-dimethylsuccinic anhydride (38.4 g.), hydrindene (36 g.) and aluminium chloride (52 g.) in nitrobenzene (150 c.c.) exactly as in the case of β-4-hydrindoylpropionic acid. The crude product was crystallised from acetic acid and the first crop recrystallised from alcohol (charcoal) as colourless prisms, m.p. 139-40°, yield 15 g. No sharp melting substance could be isolated from the acetic acid mother-liquor. (Found : C, 73.1 ; H, 7.3. $C_{15}H_{18}O_3$ requires C, 73.2 ; H, 7.3 per cent).

The *methyl ester* was obtained by the action of methyl alcoholic hydrogen chloride as a colourless thick liquid, b.p. $190-91^{\circ}/6$ mm. (Found : C, 73.6; H, 7.8. $C_{16}H_{20}O_3$ requires C, 73.8; H, 7.7 per cent).

Oxidation of α -dimethyl- β -5-hydrindoylpropionic acid with alkaline permanganate was carried out exactly as in the case of β -5-hydrindoylpropionic acid and trimellitic acid (m.p. 214°) was isolated.

α -Dimethyl- γ -5-hydrindylbutyric Acid (II, R=Me).— α -Dimethyl- β -5-hydrindoylpropionic acid (8 g.) was reduced with amalgamated zinc (40 g.) and concentrated hydrochloric acid (40 c.c.) exactly as in the preparation of γ -5-hydrindylbutyric acid. The reduced solid was purified by extraction with sodium carbonate solution. It crystallised from petroleum ether (b.p. $40-60^{\circ}$) in colourless needles, m.p. $82-83^{\circ}$. (Found : C, 77.5; H, 8.6. $C_{15}H_{20}O_2$ requires C, 77.6; H, 8.6 per cent).

6 : 7-cycloPenteno-1-keto-2 : 2-dimethyl-1 : 2 : 3 : 4-tetrahydronaphthalene (III, R=Me).—The foregoing hydrindylbutyric acid (7 g.) was cyclised by heating with sulphuric acid (21 c.c.) and water (7 c.c.). The product was obtained as a colourless oil, b.p. $170^{\circ}/10$ mm., yield 5.4 g. (85% of theory). (Found : C, 83.8; H, 8.5. $C_{15}H_{18}O$ requires C, 84.1; H, 8.4 per cent).

Oxidation of the foregoing keto-cyclic compound (III, R=Me) to benzene-1 : 2 : 3 : 4-tetracarboxylic acid was carried out exactly in the manner described for the oxidation of 6 : 7-cyclopenteno-1-keto-1 : 2 : 3 : 4-tetrahydronaphthalene. The anhydride of the product melted at 284° .

6 : 7-cycloPenteno-2-2-dimethyl-1 : 2 : 3 : 4-tetrahydronaphthalene (IV, R=Me).—The preceding cyclic ketone (III, R=Me) was reduced by heating with amalgamated zinc (15 g.) and concentrated hydrochloric acid (20 c.c.) for 24 hours. The product was extracted with ether, the ether extract washed with water, dried and ether distilled off, when 1.7 g. of solid residue were obtained which crystallised from petrol ether (b.p. $40-60^{\circ}$) in minute prisms, m.p. 82° . (Found : C, 89.8; H, 10.1. $C_{15}H_{20}$ requires C, 90.0; H, 10.0 per cent).

Dehydrogenation of 6 : 7-cycloPenteno-2 : 2-dimethyl-1 : 2 : 3 : 4-tetrahydronaphthalene.—A mixture of the hydrocarbon (1 g.) and selenium (1.5 g.) was heated in a sealed tube at $300-320^{\circ}$ for 24 hours. The product was thoroughly extracted with ether which left a slightly coloured solid on evaporation. This distilled over sodium under reduced pressure as a colourless solid (0.6 g.). The hydrocarbon, thus obtained, on being washed with a concentrated alcoholic solution of picric acid (0.6 g.) gave a picrate which

after recrystallisation melted at $107-108^{\circ}$. (Found : C, 58.2 ; H, 4.2. $C_{20}H_{17}O_7N_3$ requires C, 58.4 ; H, 4.1 per cent). The hydrocarbon was regenerated from the picrate by distribution between ammonia and ether. The ether solution was washed with water and the solvent evaporated. The hydrocarbon, thus obtained, crystallised from methyl alcohol in beautiful shining flakes, m.p. 104° . (Found : C, 92.3 ; H, 7.7. $C_{14}H_{14}$ requires C, 92.3 ; H, 7.7 per cent).

The author desires to express his grateful thanks to Dr. J. C. Bardhan for his kind interest in this investigation and to Dr. M. Q. Khuda for the facilities he has given for carrying out this investigation.

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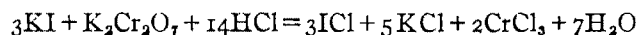
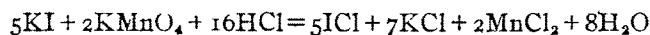
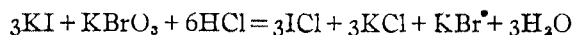
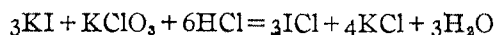
Received January 9, 1939

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS. PART V. OXIDATION WITH POTASSIUM CHLORATE.

By BALWANT SINGH AND SOHAN SINGH.

Bromate, iodate, permanganate and dichromate of potassium were determined potentiometrically, using a platinum electrode coupled with a saturated calomel electrode. A known weight of each substance was mixed with a known excess of potassium iodide and the required amount of hydrochloric acid to keep its concentration above 5*N*. The excess of potassium iodide was determined by titrating the mixture against standard potassium chlorate. The titrations were conducted at 10° in an atmosphere of carbon dioxide. At the equivalence point there was a sharp jump in potential in each case.

In presence of an excess of hydrochloric acid, potassium iodide reacts with chlorate, bromate, iodate, permanganate and dichromate of potassium. The reactions are represented by the equations :—



These reactions have been made use of in the quantitative estimation of bromate, iodate, permanganate and to dichromate of potassium.

EXPERIMENTAL.

A known weight of each substance was mixed with a known excess of potassium iodide solution and the required amount of hydrochloric acid to keep its concentration above 5*N*. The excess of potassium iodide was determined by titrating the mixture potentiometrically against standard potassium chlorate. The titrations were conducted at 10° in an atmosphere of carbon dioxide and a micro-burette was used to add the standard solution near the equivalence point.

The indicator electrode, which consisted of a bright platinum foil, immersed in the solution to be titrated, was coupled with saturated calomel electrode. A series of potentiometric titrations were performed with different amounts of each substance. One titration, as typical of that set, is recorded in the following table

TABLE I.

0.7797 G. of KI and 0.1458 g. of KMnO_4 , dissolved in 20 c. c. of water and 50 c. c. of conc. HCl acid titrated against $M/30\text{-KClO}_3$.

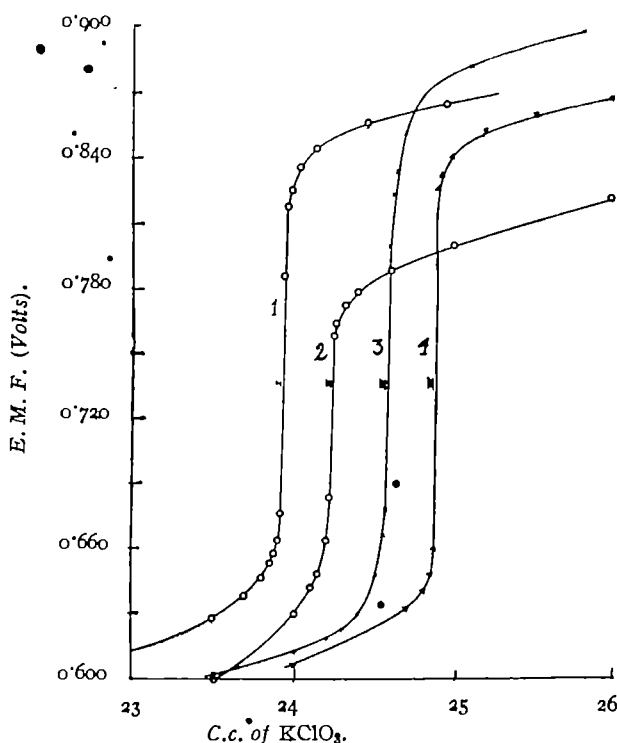
KClO_3	E.M.F.	E/C	KClO_3	E.M.F.	E/C
		(m. volts/c. c.)			(m. volts/c. c.)
0.000 c. c.	0.326 volts		23.500 c. c.	0.628 volts	
0.200	0.336	50	23.700	0.638	50
0.500	0.380	147	23.800	0.646	80
1.000	0.464	168	23.850	0.653	140
2.000	0.492	28	23.875	0.658	200
4.000	0.520	14	23.900	0.664	240
7.000	0.540	7	23.925	0.676	480
10.000	0.556	5	23.950	0.786	4400 (maximum)
13.000	0.564	3	23.975	0.818	1280
16.000	0.572	3	24.000	0.826	320
19.000	0.580	3	24.050	0.836	200
22.000	0.590	3	24.150	0.844	80
23.000	0.612	22	24.450	0.856	40
		32	24.950	0.864	16

The titrations, one for each substance, are represented by the curves given in Fig. 1.

DISCUSSION.

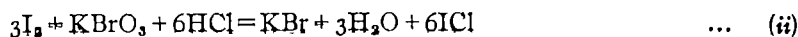
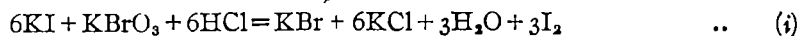
For these titrations, with the first few additions of the titrant, the E.M.F. rose abruptly except in a few cases. This was followed by a steady increase in potential till the equivalence point with further additions of the reagent. At the equivalence point, there was a jump in potential in

FIG. 1.

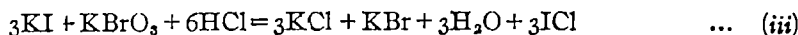


each case. For the addition of 0.025 c. c. of the titrant, the inflection potential was of the order of 70, 125, 110 and 166 m. volts for potassium bromate, potassium iodate, potassium permanganate and potassium dichromate. After the equivalence point, there was again a steady rise in the potential on further addition of the reagent.

All these reactions take place in two stages. The two stages of the reaction in the case of potassium iodide and potassium bromate in the presence of a large excess of hydrochloric acid, for example, are as follows :—



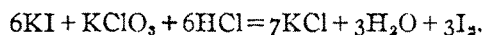
and the complete reaction is represented by a single equation as :



In all these titrations, an excess of potassium iodide (according to the complete reaction equation) was added to each of the oxidants, viz.,

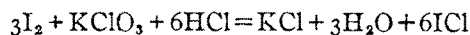
KBrO_3 , KIO_3 , KMnO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$. This amount of the iodide may or may not be in excess to that required by the oxidant according to the first stage reaction equation.

When the amount of the iodide was in excess to that required by the oxidant, the unreacted iodide was oxidised to iodine on the addition of the titrant (KClO_3).



When all the iodide was oxidised to iodine, the first break in potential was observed.

When the liberated iodine was converted into iodine monochloride on further addition of the titrant,



another break, much larger in magnitude than the first one, took place. This second jump in the E.M.F. corresponded to the equivalence point in the titration.

When the amount of the iodide was not in excess, to that required by the oxidant (considering the first stage reaction equation), the oxidant, left after the oxidation of all the iodide to iodine, reacted with equivalent amount of the liberated iodine to form iodine monochloride. When the unreacted iodine was completely converted into iodine monochloride on the addition of the titrant, a large jump in potential corresponding to the equivalence point took place. In this case, as the iodide was completely oxidised to iodine before the addition of the titrant, only one break in E.M.F. was observed. This corresponded to the equivalence point.

In some titrations, therefore, two breaks in the E.M.F. were noticed, while in others only one was observed depending upon the amount of potassium iodide used in each titration.

From the volume of potassium chlorate corresponding to the equivalence point in each titration, the amount of each substance was calculated. In the following table, the values obtained are compared with the amounts of the substance taken.

TABLE II.

KBrO_3 taken.	Potassium bromate.		KBrO_3 found
	KI added.	KI used for KBrO_3 .	
0.2516 g	1.5010 g.	1.7494 g	0.2513 g.
0.1675	0.8271	0.4990	0.1673
0.1048	0.6176	0.3121	0.1046
0.0673	0.5092	0.2001	0.0671

TABLE II (contd.).

Potassium iodate.			
KIO ₃ taken.	KI added.	KI used for KIO ₃ .	KIO ₃ found.
0.3210 g.	1.2524 g.	0.4975 g.	0.3206 g.
0.2143	0.8304	0.3322	0.2141
0.1338	0.5133	0.2072	0.1335
0.0856	0.3326	0.1323	0.0853
0.0538	0.2342	0.0832	0.0536
Potassium permanganate.			
KMnO ₄ taken.	KI added.	KI used for KMnO ₄ .	KMnO ₄ found.
0.4831 g.	2.0286 g.	1.2682 g.	0.4824 g.
0.1931	0.8371	0.5068	0.1929
0.1458	0.7797	0.3823	0.1456
0.0892	0.4890	0.2339	0.0891
0.0425	0.3318	0.1115	0.0424
Potassium dichromate.			
K ₂ Cr ₂ O ₇ taken.	KI added.	KI used for K ₂ Cr ₂ O ₇ .	K ₂ Cr ₂ O ₇ found.
0.2431 g.	1.9221 g.	1.4107 g.	0.2432 g.
0.1951	0.6798	0.3297	0.1948
0.1446	0.4972	0.2442	0.1443
0.0963	0.4722	0.1623	0.0959
0.0484	0.2823	0.0815	0.0481

These results show that potassium bromate, potassium iodate, potassium permanganate and potassium dichromate can be determined by the potentiometric method.

The authors are indebted to the Khalsa College authorities for a research grant and for providing facilities for the work.

DEPARTMENT OF CHEMISTRY,
KHALSA COLLEGE,
AMRITSAR.

Received February 21, 1939

ACRIDINE DERIVATIVES AS ANTIMALARIALS. PART II

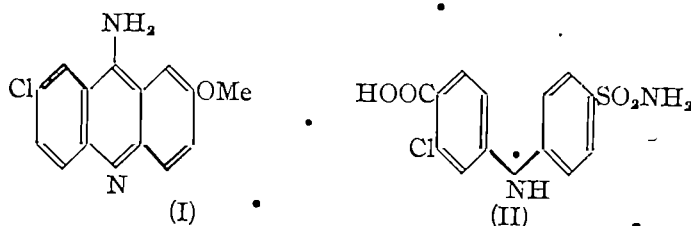
BY U. P. BASU AND S. J. DAS-GUPTA.

In order to study the influence of a nuclear sulphonamide (SO_2NH_2) substituent on the chemotherapeutic properties of 5-(dialkylaminoalkyl) aminoacridine derivatives, several sulphonamide acridines have been prepared and described. The yield of condensation product from a 5-chloroacridine derivative and an amine or any amino derivative, increases with the presence of a 2-negative substituent in the former compound. The properties of the resulting condensation products indicate that they exist in acridonimine form.

The 5-aminoacridines, and more particularly those with basic substituents in the amino group, are of therapeutic interest, as their derivatives which contain a nitro or amino group possess good antiseptic properties (*cf.* Schnitzer and Silberstein, *Z. Hyg. Infek.*, 1929, **109**, 519; Albert *et al*, *Brit. J. Expt. Path.*, 1938, **19**, 41). Those which are substituted by halogen atom in any particular position show antimalarial properties (Mauss and Meitzsch, *Klin. Woch.*, 1933, **12**, 1276; Magidson and Grigorowsky, *Ber.*, 1936, **69**, 396). As compounds with a sulphonamide grouping substituted in an aromatic amine show bacteriostatic and bactericidal action against various types of cocci, it seemed of interest to undertake the preparation of 5-(dialkylaminoalkyl) aminoacridine derivatives containing a sulphonamide grouping in the acridine nucleus. The acridine derivatives that have so far been successfully used whether as an antiseptic or as an antimalarial, contain a 2- (NH_2) in case of 'Rivanol' and Cl in case of 'Atebrin' and a 7-(ethoxy in the former and methoxy in the latter) substituent. Eisleb (*Med. u. Chem.*, 1936, **3**, 41) has further observed that 2-nitro-9-aminoacridine derivatives are also good antiseptics. Again Magidson and Grigorowsky (*loc. cit.*) have noticed that the antimalarial characteristic (as judged from the chemotherapeutic index) of a 2-nitro derivative is considerably increased if the nitro group is transferred from 2-to 3-position in the molecule. A similar shifting of the chlorine atom in 2-chloro-5 (ω -diethylaminoisoamyl)-amino-7-methoxyacridine, on the contrary, practically destroys the pharmacological characteristic of the well-known drug 'Atebrin' (*cf.* Feldman and Kopeliovich, *Arch. Pharm.*, 1935, **273**, 488). Several 2- as well as 3-sulphonamido-5-(diethylaminoalkyl) amino-7-methoxyacridine derivatives have been prepared and described in the experimental part of this paper. Their

physiological properties are being studied and would be described elsewhere.

It has been noticed that the reaction of an amine with 5-chloroacridine is much facilitated and the yield of the condensation product considerably increased if the 5-chloro acridinemolecule contains a 2-negative substituent. Such an enhanced mobility of the 5-chlorine atom has also been noticed by Eisleb (*loc. cit.*). The stability of the condensation product again seems to be dependent on the substituent at the 5-amino-nitrogen (*cf.* Choudhury, Das-Gupta and Basu, *J. Indian Chem. Soc.*, 1937, **14**, 735 ; Magidson and Grigorowsky, *loc. cit.* Drozdov, *J. Gen. Chem.*, 1936, **6**, 1641). These 5-aminoacridine derivatives are deeper in colour than their dihydrochloride salts. The latter again are readily precipitated from their aqueous solution by traces of sodium chloride. The alcoholic solutions of the bases are also more fluorescent than those of their salts. All these point to the fact that most probably these acridine derivatives or better their salts exist preferably in an isomeric acridonimine form as has been suggested by



Goodal and Kermack (*J. Chem. Soc.*, 1936, 1547), who observed the non-reactivity of 5-chloro-compound with a secondary amine (*cf.* Drozdov, *loc. cit.*). 2-Chloro-5-amino-7-methoxy acridine (I) has been found to possess all the usual characteristics of a 5-aminoacridine derivative. The compound gives no diazoreaction (*cf.* Albert and Linnel, *J. Chem. Soc.*, 1936, 1615 who found 2-chloro-5-aminoacridine to be not readily diazotisable). Albert and Linnel (*loc. cit.*) suggested the acridonimine formula for the amine.

o-Chlorobenzoic acid did not react with *p*-aminobenzene-sulphonamide under the usual conditions. 2:4-Dichlorobenzoic acid, however, gave a diphenylamine derivative (II) as usual but acridination of (II) was unsuccessful. The synthesis of 2-chloro-7-aminoacridine derivatives is in progress and it may be mentioned here that 2:5-dichloro-7-acetylaminoacridine, obtained by reacting 2:4-dichlorobenzoic acid with *p*-aminoacetanilide and by subsequent acridination, hydrolyses to 2-chloro-7-aminoacridone. The latter underwent diazotization as expected.

E X P E R I M E N T A L.

2-Sulphonamido-7-methoxy-5-dialkylaminoalkylamino-acridines.

2-Chloro-4-sulphonamido-benzoic Acid.—2-Chloro-4-sulphonamidotoluene was prepared by reacting *o*-chlorotoluene and chlorosulphonic acid (1 : 5) at a low temperature and finishing the reaction by heating the mixture at 60° for 2 hours. The resulting mixture was poured over ice and the semi-solid mass was directly treated with liquor ammonia, when a crystalline product was obtained, which was recrystallised from benzene, m. p. 121-23°. (Found : N, 7.06. $C_7H_8O_2NCIS$ requires N, 6.81 per cent). 2-Chloro-3-sulphonamidotoluene has m. p. 128° (*cf.* Wynne, *J. Chem. Soc.*, 1892, 61, 1036).

The sulphonamidotoluene (1 mol.) was refluxed with a 10% solution of potassium permanganate (2.2 mol.) till the colour of permanganate was discharged. After the removal of manganese dioxide the filtrate was acidified with hydrochloric acid and evaporated to a small volume and allowed to cool. 2-Chloro-4-sulphonamidobenzoic acid crystallised out in fine needles, m.p. 198-99°. (Found : N, 6.04. $C_7H_6O_4NCIS$ requires N, 5.94 per cent).

5-Sulphonamido-4'-methoxydiphenylamine-2-carboxylic Acid.—A mixture of 2-chloro-4-sulphonamidobenzoic acid (2.3 g.), *p*-anisidine (1.2 g.), anhydrous potassium carbonate (1.4 g.), copper powder (0.1 g.) and amyl alcohol (25 c. c.) was refluxed together for about 5 hours. Most of the amyl alcohol was removed by distillation and the residual mass after dilution with water was steam-distilled to remove the last traces of amyl alcohol and anisidine. It was then filtered hot (charcoal), cooled and acidified with dilute hydrochloric acid. The diphenylamine-carboxylic acid, thus isolated, was crystallised from dilute alcohol and obtained in greenish yellow needles, m.p. 242°. (Found : N, 8.62. $C_{11}H_{14}O_3N_2S$ requires N, 8.7 per cent).

2-Sulphonamido-5-chloro-7-methoxyacridine.—The above diphenylamine-carboxylic acid was heated with excess of phosphorus oxychloride on a boiling water-bath for 2 hours. The excess of phosphorus oxychloride was removed *in vacuo*. The residual mass was treated with ice-cold water and made alkaline with ammonia. The crude chloroacridine, thus obtained, was crystallised from acetone in small yellow needles, decomposing slowly above 240°, yield theoretical. (Found : N, 8.48. $C_{14}H_{11}O_3N_2ClS$ requires N, 8.68 per cent). The compound is soluble in both acids and alkalis. It gives feeble fluorescence in dilute alcoholic solution.

2-Sulphonamido-7-methoxy-5-(ω -diethylamino-isoamyl)-aminoacridine.—The above chloroacridine (1 g.) was dissolved in phenol and heated with

δ -diethylamino- α -methylbutylamine (0.7 g.) for about 1 hour. The reaction mixture was poured into sodium hydroxide solution. On stirring a clear solution was obtained. This was extracted with ether several times and made faintly alkaline by adding dilute hydrochloric acid. A viscous mass separated, which solidified on washing with cold water. It was dissolved in dilute acetic acid, the acid solution was filtered, extracted with ether and made alkaline with ammonia. The base, thus obtained, was collected and dried. It crystallised from acetone-petroleum ether mixture (1:4) in orange-yellow crystals, m.p. 115-120°. (Found: N, 12.10; S, 7.52. $C_{23}H_{32}O_3N_4S$ requires N, 12.61; S, 7.21 per cent). The substance is extremely bitter in taste and is highly soluble in alcohol, acids and dilute alkalis. Its alcoholic solution exhibits emerald-green fluorescence. In acids the fluorescence is weaker.

2-Sulphonamido-7-methoxy-5-(δ -diethylaminobutyl)aminoacridine.—2-Sulphonamido-5-chloro-7-methoxyacridine (1 g.) dissolved in phenol was heated with δ -diethylaminobutylamine (0.6 g.). The reaction mixture was subsequently treated as in the previous case and the base was finally crystallised from acetone-petroleum ether (1:3) mixture in yellow microscopic needles, m.p. 110-115°. (Found: N, 12.82. $C_{22}H_{30}O_3N_4S$ requires N, 13.02 per cent. It is also highly soluble in alcohol and difficultly soluble in ether. It resembles the previous compound in the fluorescence of its alcoholic and acid solutions.

3-Sulphonamido-7-methoxy-5-dialkylaminoalkyl-aminoacridines.

2-Chloro-5-sulphonamidobenzoic Acid.—*o*-Chlorobenzoic acid (1 mol.) was slowly added to chloro sulphonic acid (5 mol.) cooled in ice. The mixture was gradually heated up to 90-95° and kept at that temperature for 1½-2 hours. It was finally poured into crushed ice with stirring when the sulphonyl chloride derivative (m.p. 101° on crystallisation from petroleum ether) separated. It was dissolved in strong ammonia and the solution on acidification gave 2-chloro-5-sulphonamidobenzoic acid which crystallised from dilute alcohol in colourless needles, m.p. 217-19°. (Found: N, 6.12. $C_7H_5O_4NCIS$ requires N, 5.94 per cent).

2-Chloro-5-sulphondiethylamidobenzoic Acid.—The sulphonyl chloride, obtained in the previous experiment, when treated with diethylamine and the resulting solution acidified gave the acid in colourless needles, m.p. 147° after crystallisation from benzene. (Found: N, 4.91. $C_{11}H_{14}O_4NCIS$ requires N, 4.8 per cent).

2-Chloro-5-sulphonphenylamidobenzoic Acid.—The sulphonyl chloride with aniline gave after isolation in the usual manner 2-chloro-5-sulphon-

phenylamidobenzoic acid, m.p. $194-95^{\circ}$ after crystallisation from dilute alcohol in colourless needles. (Found : N, 4.67. $C_{13}H_{10}O_4NClS$ requires N, 4.49 per cent).

4-Sulphonamido-4'-methoxydiphenylamine-2-carboxylic Acid.—2-Chloro-5-sulphonamidobenzoic acid (2.3 g.), *p*-anisidine (1.2 g.), anhydrous potassium carbonate (1.4 g.) and copper powder (0.1 g.) were refluxed in amyl alcohol for several hours. After treatment of the reaction product in the way as described under 5-sulphonamido-4'-methoxydiphenylamine-2-carboxylic acid, the crude product was isolated as a semi-solid mass. This was finally crystallised from dilute alcohol (charcoal) in greenish yellow needles, m.p. $239-40^{\circ}$. (Found : N, 8.53. $C_{14}H_{14}O_5N_2S$ requires N, 8.7 per cent).

4-Sulphondiethylamido-4'-methoxydiphenylamine-2-carboxylic Acid.—2-Chloro-5-sulphondiethylamidobenzoic acid, when reacted with *p*-anisidine under the above conditions, afforded the related diphenylamine-carboxylic acid, m.p. $170-71^{\circ}$ after crystallisation from dilute alcohol. (Found : N, 7.51. $C_{18}H_{22}O_5N_2S$ requires N, 7.4 per cent).

4-Sulphonphenylamido-4'-methoxydiphenylamine-2-carboxylic Acid.—2-Chloro-5-sulphonphenylamidobenzoic acid, when treated with *p*-anisidine under the usual conditions, afforded the diphenylamine derivative which after crystallisation from rectified spirit (charcoal) in fine yellow needles had m.p. 233° . (Found : N, 7.22. $C_{20}H_{18}O_5N_2S$ requires N, 7.04 per cent).

3-Sulphonamido-5-chloro-7-methoxyacridine.—4-Sulphonamido-4'-methoxydiphenylamine-2-carboxylic acid was heated with excess of phosphorus oxychloride and the reaction mixture was subsequently treated in the way as described in the preparation of 2-sulphonamido-5-chloro-7-methoxyacridine. The product crystallised from acetone in yellow needles decomposing slowly above 230° . (Found : Cl, 10.95. $C_{14}H_{11}O_3N_2ClS$ requires Cl, 11.01 per cent). The yield is about 80-90 % of the theory. It is soluble in dilute acids and alkalis.

3-Sulphondiethylamido-5-chloro-7-methoxyacridine.—4-Sulphondiethylamido-4'-methoxydiphenylamine-2-carboxylic acid when reacted with phosphorus oxychloride afforded the above acridine derivative. It crystallises from benzene (charcoal) in yellow microscopic needles, m.p. $184-86^{\circ}$. (Found : N, 7.51. $C_{18}H_{19}O_3N_2ClS$ requires N, 7.39 per cent). It is soluble in dilute acids but insoluble in dilute alkalis. The compound is fluorescent in solution.

3-Sulphonphenylamido-5-chloro-7-methoxyacridine was also prepared in the same way by treating 4-sulphonphenylamido-4'-methoxydiphenylamine-2-carboxylic acid with phosphorus oxychloride. It separates from

acetone as yellow needles, m.p. 207-208°. (Found : N, 7.06. $C_{20}H_{15}O_3N_2ClS$ requires N, 7.02 per cent). It is again soluble both in acids and alkalis.

3-Sulphonamido-7-methoxy-5-(ω-diethylamino-isoamyl)aminoacridine.—3-Sulphonamido-5-chloro-7-methoxyacridine on treatment with δ-diethyl-amino-α-methylbutylamine as previously described in the case of 2-sulphon-amido compound, afforded the above sulphonamidoacridine. It crystallised from a mixture of acetone and petroleum ether in the cold in yellow needles, m.p. indefinite, ca 135°. (Found : N, 12.29. $C_{23}H_{32}O_3N_4S$ requires N, 12.61 per cent). It is soluble in dilute acids and alkalis, slightly soluble in ether but freely in alcohol. The hydrochloride has less intense fluorescence than the free base.

3-Sulphonamido-7-methoxy-5-(δ-diethylaminobutyl) aminoacridine.—The foregoing chloroacridine derivative on treatment with δ-diethylamino-butylamine gave the 5-aminoacridine derivative which separated from acetone-petroleum ether mixture in orange-yellow needles, melting indefinitely at 138°. (Found : N, 12.76 ; S 7.71. $C_{22}H_{30}O_3N_4S$ requires N, 13.02 ; S 7.44 per cent). It is bitter in taste and possesses all the usual characteristics of the 5-aminoacridine derivatives.

3-Sulphondiethylamido-7-methoxy-5-(ω-diethylaminoisoamyl) aminoacridine.—The corresponding 5-chloro derivative with the δ-diethylamino-α-methylbutylamine gave this amino derivative as a low melting solid. Accordingly this was extracted with ether, ethereal solution dried over anhydrous potassium carbonate, and treated with dry hydrogen chloride. The dihydrochloride, thus obtained, was collected and crystallised from alcohol-ether mixture (1 : 2) as a yellow crystalline powder, m.p. 240-41°. (Found : N, 9.66 ; Cl, 12.12. $C_{27}H_{40}O_3N_4S$, 2 HCl requires N, 9.77 ; Cl, 12.39 per cent). The *dihydrochloride* is extremely soluble in water and alcohol and bitter in taste. The base is more coloured than this hydrochloride, the latter being less fluorescent in solution than the former.

3-Sulphondiethylamido-7-methoxy-5-(δ-diethylaminobutyl) aminoacridine.—3-Sulphondiethylamino-5-chloroacridine derivative with δ-diethylamino-butylamine also afforded the base as usual. The product was converted into the dihydrochloride as in the former case, which was finally crystallised from a mixture of alcohol and ether in canary yellow needles, m.p. 251-52°. (Found : N, 9.97 ; Cl, 12.59. $C_{26}H_{38}O_3N_4S$, 2HCl requires N, 10.02 ; Cl, 12.7 per cent). In this case also the base is more coloured and imparts more fluorescence to its alcoholic solution. This base is highly soluble in ether as well as in alcohol.

3-Sulphonphenylamido-7-methoxy-5-(ω-diethylaminoisoamyl) aminoacridine.—The compound was obtained from the corresponding 5-chloro

acridine derivative and the appropriate amine. It separates from acetone-petroleum ether mixture in orange-yellow crystals, melting indefinitely at $105-110^{\circ}$. (Found : N, 10.59. $C_{26}H_{18}O_3N_4S$ requires N, 10.77 per cent).

3-Sulphophenylamido-7-methoxy-5-(ω -diethylaminobutyl) aminoacridine.—It was isolated in the usual manner and found to separate from acetone-petroleum ether in orange-yellow needles melting indefinitely at $100^{\circ}-105^{\circ}$. (Found : N, 10.87 ; S, 6.7. $C_{28}H_{34}O_3N_4S$ requires N, 11.07 ; S, 6.32 per cent) The compound is soluble in acids, alkalis, alcohol and ether, and is highly fluorescent in solution

5-Chloro-4'-sulphonamidodiphenylamine-4-carboxylic Acid.—Equimolecular quantities of 2:4-dichlorobenzoic acid and *p*-aminobenzenesulphonamide under the usual conditions afforded the foregoing substance. Crystallised from dilute alcohol (charcoal) in greenish-yellow needles it had m.p. 207° . (Found : N 8.29. $C_{13}H_{11}O_4N_2ClS$ requires N, 8.57 per cent). The compound was heated with phosphorus oxychloride, and with a mixture of the latter and phosphorus pentachloride at various temperatures up to 150° , but no acridination took place. Similarly 2:4-dichlorobenzoic acid and *p*-aminobenzenesulphonamide gave 5-chloro-4'-sulphondiethylamidodiphenylamine-4-carboxylic acid, m.p. $101-102^{\circ}$. (Found : N 7.07 ; Cl, 9.41. $C_{17}H_{19}O_4N_2ClS$ requires N, 7.47 ; Cl, 9.48 per cent). The acid also resisted acridination.

5-Chloro-4'-acetylamidodiphenylamine-2-carboxylic Acid.—The 2:4-dichlorobenzoic acid and *p*-aminoacetanilide in molecular proportions under the usual conditions afforded the related diphenylamine-carboxylic acid, m.p. $287-288^{\circ}$, after crystallisation from a large volume of alcohol. (Found : N, 8.96. $C_{15}H_{13}O_3N_2Cl$ requires N, 9.19 per cent).

2:5-Dichloro-7-acetylaminoacridine.—The above compound on treatment with phosphorus oxychloride afforded the chloroacridine in fine orange needles, m.p. $242-243^{\circ}$ after crystallisation from benzene. (Found : N 8.91. $C_{15}H_{10}ON_2Cl_2$ requires N, 9.18 per cent).

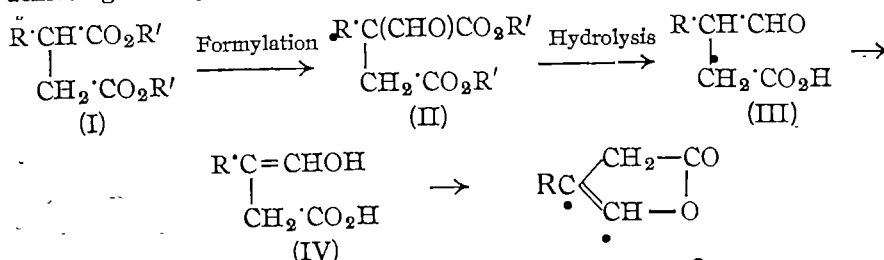
2-Chloro-7-aminoacridone.—The foregoing chloroacridine (0.5 g.) was boiled with 15 c.c. of hydrochloric acid (24 %) for $\frac{1}{2}$ hour. The reaction mixture was diluted with water, cooled and the solid was triturated with ammonia and filtered. The aminoacridone was crystallised from alcohol in dark red needles, decomposing above 250° . (Found : Cl, 14.3. $C_{13}H_9ON_2Cl$ requires Cl, 14.51 per cent). It is sparingly soluble in ether and benzene, but is soluble in acids and gives a diazo reaction.

EXPERIMENTS TOWARDS THE SYNTHESIS OF PHYSIO-
LOGICALLY ACTIVE LACTONES. PART I. *cyclo*-
PENTYL- AND *cyclo*HEXYLSUCCINIC ACIDS.
RESOLUTION OF *dl-cyclo*PENTYL-
SUCCINIC ACID.*

BY S. K. RANGANATHAN.

The syntheses of α -ring substituted succinic acids, such as α -*cyclopentyl*-, α -*cyclohexyl*succinic acid, required in connection with the synthesis of physiologically active lactones, are described. Some preliminary experiments towards the conversion of ethyl α -*cyclopentyl*succinate into the requisite lactonic structure have been made, and β -aldehyde β -*cyclopentyl*propionic acid has been obtained. *dl-cyclo*Pentylsuccinic acid has been resolved into its optical antipodes.

Correlating the structural features of the cardiac aglucones with their physiological property, it can be inferred that the latter effect is mainly due to the presence of a $\Delta^{\beta\gamma}$ -unsaturated γ -lactone ring in the molecule. The aetiocholane ring structure perhaps serves the purpose of a convenient framework for the attachment of the lactone group. It was considered of interest to verify this hypothesis by the synthesis of lactones of relevant structure carrying in the first instance simpler rings like *cyclopentane* and *cyclohexane* in place of the aetiocholane ring. The following scheme was drawn up for achieving the object in view.



(R = *cyclopentyl*, *cyclohexyl*; R' = H, Et).

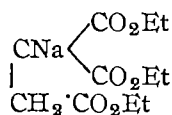
The last two stages follow the postulative mechanism of Windaus (*Wiss. Math. Phys. Kl., Göttingen*, 1835, 70) in his derivation of the lactone structure of the cardiac aglucones from the side chain of *nor*cholanic acid.

In the present paper is described the synthesis of (I, R = *cyclopentyl*, *cyclohexyl*), (II, R = *cyclopentyl*) and (III, R = *cyclopentyl*) reserving for a future communication a fuller study of (II) and (III) and the attempts made

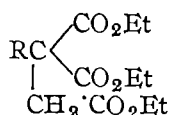
* A preliminary note on this paper appeared in *Current Science*, 1937, 6, 277.

** *cyclopentyl* and *cyclohexyl* have been expressed by c.p. and c.h.

to convert them into the lactone (V). We at first found the need for a standard method for the synthesis of acids represented by (I). The Reformatsky reaction of ethyl α -bromosuccinate with cyclopentanone as a first stage in the synthesis of α -cyclopentylsuccinic acid (I, R = c.p.; R' = H) was tried but due to the unsatisfactory nature of the condensation, the method had to be abandoned. A convenient method consisted in condensing cyclopentyl bromide with ethyl sodioethane- $\alpha\alpha\beta$ -tricarboxylate (V) (Bischoff, *Annalen*, 1882, 214, 38) to furnish in good yield ethyl α -cyclopentylethane- $\alpha\alpha\beta$ -tricarboxylate (VI, R = c.p.). The latter on hydrolysis with hydrochloric acid,



(V)



(VI, R = c.p., c.h.)

followed by decarboxylation, gave α -cyclopentylsuccinic acid (I, R = c.p.; R' = H). cycloHexyl bromide also condensed with (V), in lesser yield, to give ethyl α -cyclohexylethane- $\alpha\alpha\beta$ -tricarboxylate (VI, R = c.p.), from which α -cyclohexylsuccinic acid (I, R = c.h.; R' = H) was obtained. The anhydrides of the two acids have been prepared, and characterised.

Ethyl α -cyclopentylsuccinate (I, R = c.p.; R' = Et) was reacted with ethyl formate and sodium (Wislicenus, Böklen, Reuthe, *Annalen*, 1908, 363, 347; Carrière, *Ann. chim. phys.*, 1922, ix, 17, 42) when ethyl α -aldehydo- α -cyclopentylsuccinate (II, R = c.p.; R' = Et) was formed in 35% yield. On hydrolysis with dilute hydrochloric acid this was transformed into the original α -cyclopentylsuccinic acid. It was found to dissolve in cold alkali, and could be reprecipitated unchanged. It did not give a phenylurethane and was found unchanged after heating in a sealed tube with water at 130° during 3 hours. This latter property is in direct contrast to ethyl formylsuccinate which decomposed under similar circumstances to yield β -aldehydopropionic acid (Wislicenus *et. al.*, *loc. cit.*). Conversion into β -aldehydo- β -cyclopentylpropionic acid (III, R = c.p.) (isolated as semicarbazone) was, however, possible on prolonged boiling with dilute oxalic acid (*cf.* Carrière, *loc. cit.*). A trace of copper-bronze was found to facilitate this reaction. A fuller study is being made of these compounds.

In view of the work of Wren and Crawford (*J. Chem. Soc.*, 1937, 230) it was considered of interest to resolve the synthesised *dl*- α -cyclopentylsuccinic acid. The neutral brucine salt was formed, and resolution effected in the usual way. The *d* and *l* forms had $[\alpha]_D^{20}$, +17.81° and $[\alpha]_D^{20}$, -16.94° respectively. They melt at 135°, while the *dl*-acid melts at 116-117°.

EXPERIMENTAL.

cyclopentyl Bromide.—The method indicated by Hiers and Adams (*J. Amer. Chem. Soc.*, 1926, **48**, 2389) has been applied for the preparation of this compound. *cyclopentanol* (146 g.) was cooled to -5° under good stirring. Pure phosphorus tribromide (200 g.) was added slowly and care was taken to see that the temperature did not rise above 0° . The mixture was stirred at room temperature for 2 hours, after which it was gradually raised to 100° and the mixture stirred for 1 hour. On cooling, the product was extracted thrice with petroleum ether, and the extract repeatedly washed with water, dried over anhydrous calcium chloride, and the solvent removed. *cyclopentyl bromide* distilled at $133-34^{\circ}/680$ mm., yield 200 g. (88%). The above method would appear to be by far the best one for the preparation of the bromide.

Ethyl α -cyclopentylethane- $\alpha\beta$ -tricarboxylate (VI, $R=c.p.$).—Sodium (28.75 g.) was dissolved in absolute alcohol (400 c.c.) and after cooling ethyl ethane- $\alpha\beta$ -tricarboxylate (308 g.; b.p. $170-72^{\circ}/27$ mm.; Bischoff. *loc. cit.*) was added gradually, followed by *cyclopentyl bromide* (186 g.). There was no immediate reaction, but it started after $\frac{1}{2}$ hour's heating on a water-bath, with deposition of sodium bromide. The heating was continued for 12-14 hours after which the product was decomposed with a large excess of water. The oily layer (in which the smell of *cyclopentene* was apparent) was taken up in ether, the ethereal solution washed with water, dried and ether removed. The ethereal residue was fractionated in *vacuo*, 25 g. distilled below $165^{\circ}/10$ mm., and the major fraction representing ethyl α -cyclopentyl-ethane- $\alpha\beta$ -tricarboxylate distilled at $165-170^{\circ}/5$ mm. as a colourless oil with feeble odour. On redistillation it had b.p. $166^{\circ}/5$ mm., yield 300 g. (75 %). (Found: C, 61.0; H, 8.1. $C_{18}H_{26}O_8$ requires C, 61.1; H, 8.3 per cent).

Hydrolysis, followed by Decarboxylation of (VI, $R=c.p.$) with *Hydrochloric Acid*: α -cyclopentylsuccinic Acid (I, $R=c.p.$; $R'=H$).—The above ester (VI, $R=c.p.$) was mixed with hydrochloric acid (d 1.19, 400 c.c.); and was refluxed on a sand-bath for 70 hours, a further addition of hydrochloric acid (50 c. c.) being made towards the end of this period. On allowing to cool, α -cyclopentylsuccinic acid separated in an almost pure condition. α -cyclopentylsuccinic acid crystallises from boiling water in plates, m. p. $116-17^{\circ}$, yield 70 g. It is sparingly soluble in cold water, and easily soluble in the common organic solvents except ligroin. [Found: C, 58.1; H, 7.9; Equiv., 93.8. $C_9H_{14}O_4$ (dibasic) requires C, 58.1; H, 7.5 per cent. Equiv., 93).

Alkaline hydrolysis of (VI, $R=c.p.$) invariably gave rise to a mixture

of the corresponding tricarboxylic acid* (not isolated in pure state) and an ester-acid, m. p., 112° . In this latter compound only two of the ester groups have been hydrolysed. [Found: Equiv., 128.5. $C_{12}H_{18}O_6$ (dibasic) requires Equiv., 129].

Anhydride of α -cyclopentylsuccinic Acid.—The acid (3 g.) was mixed with freshly distilled acetyl chloride (6 c. c.) and warmed on the water-bath for 90 minutes. The excess of acetyl chloride was then removed under vacuum and the residual anhydride distilled. α -cyclopentylsuccinic anhydride boiled at $120-125^{\circ}/3$ mm. ($176^{\circ}/30$ mm.) as a thick oil. (Found: C, 64.0; H, 7.3. $C_9H_{12}O_3$ requires C, 64.3; H, 7.1 per cent).

The *semi-p-toluidide- α -cyclopentylsuccinic acid* was formed on mixing theoretical amounts of the above anhydride and *p*-toluidine in benzene solution. It crystallises in plates from dilute alcohol, m. p. 174° . (Found: N, 5.2. $C_{16}H_{18}O_3N$ requires N, 5.1 per cent).

Ethyl α -cyclopentylsuccinate (I, $R=c.p.$; $R'=Et$)— α -cyclopentylsuccinic acid (50 g.) was dissolved in absolute alcohol (300 c. c.) and to the solution concentrated sulphuric acid (6.5 c. c.) was added. The mixture was heated under reflux on steam-bath for 6 hours. The major portion of the alcohol was then removed, and the product decomposed with water. The ester that separated was extracted with ether, the ethereal solution washed with sodium bicarbonate solution, water, dried and ether removed. Ethyl α -cyclopentylsuccinate distilled as a colourless thick oil, b. p., $120-125^{\circ}/2-4$ mm. On redistillation it had b. p. $120^{\circ}/2$ mm.; d_{25} , 1.0303. (Found: C, 64.2, H, 9.3. $C_{13}H_{22}O_4$ requires C, 64.5; H, 9.1 per cent).

Ethyl α -Aldehyde- α -cyclopentylsuccinate (II, $R=c.p.$; $R'=Et$). The improved method of Carrière (*loc. cit.*) was adopted for formylation and isolation of the requisite ester. Sodium (7.2 g.) was suspended in anhydrous ether (250 c. c.) and then absolute alcohol (14.4 g.) was added. The product was allowed to stand overnight to ensure complete formation of sodium ethoxide. A mixture of freshly distilled ethyl formate (23 g.) and ethyl α -cyclopentylsuccinate (60 g.) was then added to it when a fairly vigorous reaction set in, subsiding after a few hours. After standing for 3 days, the resulting product was decomposed carefully with 50% sulphuric acid (48 g.) and ice (75 g.). The ethereal layer was separated, and the aqueous layer extracted with ether. The total ethereal solution was shaken with 50% potassium carbonate solution (175 c. c. in 3 equal lots). The formyl compound was dissolved in potassium carbonate solution while the unreacted ethyl α -cyclopentylsuccinate remained insoluble. The potassium carbonate extract was acidified cautiously with dilute sulphuric acid. The ester that separated was taken up in ether, the ethereal solution

washed with water, dried, ether removed and the residual oil distilled in vacuum. Ethyl α -aldehydo- α -cyclopentylsuccinate boiled at $150-154^{\circ}/3-5$ mm. as a thick, viscid oil with characteristic odour, yield 23 g. It gave a purple colouration with alcoholic ferric chloride solution. It did not furnish a phenylurethane with phenyl isocyanate. On being shaken with aqueous caustic potash (2 mol. 10%), it dissolved with evolution of heat, but was reprecipitated on acidification (even after one hour's standing). (Found: C, 62.4; H, 8.3. $C_{14}H_{22}O_5$ requires C, 62.2; H, 8.15 per cent).

Behaviour of the above Ester with dilute Hydrochloric Acid.—The ester (1 g.) was mixed with concentrated hydrochloric acid (4 c.c.) and water (8 c.c.) and refluxed on a sand-bath till the ester went into solution. On allowing to cool, a solid was obtained, which after one crystallisation from water, melted at $116-117^{\circ}$. It has been identified as α -cyclopentylsuccinic acid by a mixed melting point determination.

Attempt towards Decarboxylation.—Following Wislicenus *et. al* (*loc. cit.*) the above ester (1 g.) was mixed with water (5 c.c.) and heated in a sealed tube at 130° for 3 hours. On reopening the tube, an oil was obtained, b. p. $145-50^{\circ}/2$ mm., but this was, however, identified as the original ester itself by direct comparison and analysis. (Found: C, 62.3; H, 8.4. $C_{14}H_{22}O_5$ requires C, 62.2; H, 8.15 per cent).

β -Aldehydo- β -cyclopentylpropionic Acid.—The ester (II, R=c.p.; R'=Et) described above (4 g.) was suspended in a solution of oxalic acid (3 g.) in water (30 c.c.) and copper-bronze (0.5 g.) added to it. The mixture was heated under reflux for 6 hours, at the end of which the unreacted ester was decanted. The aqueous layer was extracted with ether, the ethereal solution dried and ether removed. The residue was treated with semicarbazide hydrochloride and sodium acetate in aqueous solution when after a time the semicarbazone separated, yield 0.9 g. The semicarbazone crystallised from dilute alcohol in prisms, m.p. 200° . Analysis reveals that it is the semicarbazone of β -aldehydo- β -cyclopentylpropionic acid. (Found: C, 52.3; H, 7.2. $C_{10}H_{17}O_3N_3$ requires C, 52.8; H, 7.5 per cent). Experiments towards the isolation of this compound in large amounts are in progress.

cycloHexyl Bromide.—The procedure adopted for the preparation of cyclopentyl bromide was followed, b.p. $159-160^{\circ}/680$ mm, yield 143 g. of the bromide (88%) from 100 g. of cyclohexanol and 108 g. of phosphorus tribromide.

Ethyl α -cycloHexylethane- $\alpha\beta$ -tricarboxylate (VI, R=c.h.).—The procedure described for the preparation of the cyclopentyl analogue was followed: [cycloHexyl bromide (109 g.), ethyl ethane- $\alpha\beta$ -tricarboxylate (164 g.), sodium (15.3 g.), and alcohol (225 c.c.)]. Ethyl α -cyclohexylethane- $\alpha\beta$ -tricarboxylate boiled at 150–165°/0.5 mm. Redistillation afforded the pure compound, b.p. 160°/2 mm., yield 56 g. (25 %). The unreacted low boiling fractions consisted of unreacted cyclohexyl bromide and ethyl α -cyclohexylethane- $\alpha\beta$ -tricarboxylate. (Found : C, 61.9 ; H, 8.8. $C_{17}H_{28}O_6$ requires C, 62.19 ; H, 8.54 per cent).

α -cycloHexylsuccinic Acid (I, R=c.h. R'=H).—The above ester (37 g.) was mixed with hydrochloric acid (d 1.19, 112 c.c.) and heated under reflux on a sand-bath. Hydrolysis was slow, and complete decarboxylation took a longer time. After heating for 96 hours, the product was cooled when α -cyclohexylsuccinic acid crystallised out, yield 15 g.. It was recrystallised from boiling water in colourless plates, m.p. 145°. It is sparingly soluble in cold water, benzene and chloroform and easily soluble in acetone and alcohol. [Found : C, 59.6 ; H, 8.1 ; Equiv., 99.5. $C_{10}H_{16}O_4$ (dibasic) requires C, 60.0 ; H, 8.1 per cent. Equiv., 100].

α -cycloHexylsuccinic Anhydride.—The above acid (3 g.) was refluxed with freshly distilled acetyl chloride (6 c.c.) on the water-bath for 2½ hours, after which the excess of acetyl chloride was removed in vacuum, and the anhydride distilled. α -cyclohexylsuccinic anhydride boils at 150°/4 mm. as a thick oil which solidifies to a crystalline mass, m.p. 42°. (Found : C, 65.7 ; H, 8.2. $C_{10}H_{14}O_3$ requires C, 65.9 ; H, 7.7 per cent).

The *semi-p-toluidide* of α -cyclohexylsuccinic acid was immediately formed on mixing equivalent amounts of *p*-toluidine, and the above anhydride in benzene solution. It crystallised from dilute alcohol in needles, m.p. 187°. (Found : N, 5.1. $C_{17}H_{23}O_3N$ requires N, 4.8 per cent).

Resolution of dl- α -cycloPentylsuccinic Acid.—*dl- α -cycloPentyl succinic acid* (m.p. 116–117°) 6.10 g., 1 mol.) was mixed with anhydrous brucine (42.4 g., 2 mol.) and the mixture dissolved in boiling water (250 c.c.). The solution was filtered hot and allowed to cool. After standing for a few hours, the solution was concentrated to 150 c.c., when 45.5 g. of salt were thrown down. In the table on page 113 is given the volume of the solvent (water) at each subsequent crystallisation, and the weight of salt recrystallised.

The brucine salt was decomposed at this stage with ammonia, and the acid liberated had $[\alpha]_D^{25} + 16.53^\circ$ ($l=1$; $c=1.996$ in acetone). To ensure that an acid of maximum rotation was obtained, this acid (2 g.) was again combined with brucine (8.5 g.) in minimum amount of water, when the salt

(1.4 g.) separated. This was recrystallised once from water (25 c.c.) and then decomposed with ammonia and the acid liberated (0.9 g.). It had $[\alpha]_D^{25} + 16.16^\circ$ ($l=1$; $c=1.484$ in acetone). On recrystallisation from water this had $[\alpha]_D^{25} + 17.81^\circ$ ($l=1$; $c=2.348$ in acetone). [Found : Equiv., 93.4. $C_9H_{14}O_4$ (dibasic) requires Equiv., 93.0].

Crystallisation.	Wt. of salt.	Vol. of solvent.
I	45.5 g.	100 c.c.
II	21.2	100
III	20.0	90
IV	14.7	60
V	10.0	50

Isolation of the laevo-isomer.—The parent mother-liquor and mother liquor from (I), (II) and (III) crystallisations were combined together and concentrated *in vacuo* to 200 c.c. ($\frac{1}{2}$ its bulk). The concentrate was cooled to 0° and the solid that separated was filtered. This process was repeated twice and the resulting mother-liquor was decomposed with ammonia and the acid liberated. This had $[\alpha]_D^{25} - 14.06^\circ$ ($l=1$; $c=2.348$ in acetone). This active acid (0.5 g.) was combined with brucine (2.1 g.) in water, the salt that separated was rejected and the active acid was liberated. This was recrystallised from water once, and then it had $[\alpha]_D^{25} - 16.94^\circ$ ($l=1$; $c=1.830$ in acetone). [Found : Equiv., 93.6. $C_{19}H_{14}O_4$ (dibasic) requires Equiv., 93.0]. Both the above active forms melted at 135° .

The author's thanks are due to Professor P. C. Guha for the interest he has taken in this work and for the kind encouragement he has given.

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Received February 17, 1939.

REVIEW

A Text-Book of Organic Chemistry.—By JAGINDAR SINGH ; WITH AN INTRODUCTION BY DR. BAWA KARTAR SINGH. PUBLISHED BY ATMA RAM & SONS, LAHORE. PRICE RS. 6-4. DEMY 16mo. PP. 800+XI.

The fact that the book has reached its third edition in four years clearly suggests that it has been well-received. Indeed, there are many things to be said in its favour. The copious illustrations, specially of industrial plants for the manufacture of the more important organic compounds, and also the coloured plates of alkaloids-bearing plants are very welcome. The author has, with marked success, struck a golden mean between an elementary and an advanced treatise on the subject and has indicated many facts of interest to Indian students.

On the other hand, treatment of the subject calls for some comments. Much of the Chapters V to XI are beyond the grasp of students in the first year Honours class of most Indian Universities and cannot successfully be taught in this class. For example, the reviewer would personally find it impossible to treat subjects like Bayer's strain theory (p. 84), Thiele's theory (p. 87), tri-valent carbon (p. 90), quinonoid structure (p. 100), steric hindrance (p. 140), in this class. A much fuller treatment than is given in the book under review, would, however, be possible in the third year class. These special chapters are dealt with separately in most of the popular textbooks and no other course seems open either to the teacher or to the student. A similar difficulty would be felt if unsaturated hydrocarbons have to be taught immediately after the saturated ones, or the use of the Grignard reagent immediately after the halogenated paraffins. There are a few printing mistakes in the book which are, however, not of a serious character. The black borders round the photographs of eminent chemists, specially of living chemists, may be omitted in the future editions.

On the whole the book is sure to prove useful and stimulating to the Honours students of the Indian Universities.

S. S. G.-S.

THE EFFECT OF THE ADDITION OF NON-ELECTROLYTES AND OF TEMPERATURE ON THE TIMES OF SETTING OF SOME TRANSPARENT INORGANIC GELS.

BY MATA PRASAD AND D. M. DESA .

The effects of the addition of non-electrolytes and temperature on the time of setting of some transparent inorganic gels prepared by the authors have been described in this paper. The heats of activation have been calculated by using Arrhenius' equation.

The addition of increasing quantities of non-electrolytes increases the time of setting of all gels, manganese arsenate gels being an exception. Increase in temperature decreases the time of setting of all gels, excepting those of sodium arsenate. The heat of activation is not found to be a characteristic property of a gel.

Several gels of inorganic substances, which have been prepared in an opaque or translucent state, have been prepared by the authors (*cf. J. Univ. Bombay*, 1938, 7, iii, 132) in a transparent condition by suitably selecting the constituents of the gel-forming mixtures and adjusting their H-ion concentration. Ceric phosphate gels were prepared for the first time.

It is known that among other conditions, the time of setting of a gel depends upon the presence of the extra amounts of non-electrolytes and upon temperature. The effect of these factors on the time of setting of the gels, referred to above, has been studied in this investigation.

The effect of the addition of non-electrolytes on the time of setting of the gels has been studied by various workers. Prasad and Hattiangadi (*J. Indian Chem. Soc.*, 1929, 6, 991) found that the addition of non-electrolytes decreases the time of setting of alkaline silicic acid gel-forming mixtures and increases that of the acidic ones. They further found that the effect of pyridine is peculiar in that it decreases the time of setting of both alkaline and acidic gel-forming mixtures. Hurd and Curver (*J. Phys. Chem.*, 1933, 7, 321) found that ammonium hydroxide, methyl-, dimethyl-, and trimethylamines, pyridine and aniline decrease the time of setting of silicic acid gels while ethyl acetate, ethyl alcohol, acetaldehyde, and acetone increase it. Canesugar and glycerine have practically no effect on the time of setting. Parmar, Mehta and Prasad (*Proc. Indian Acad. Sci.*, 1936, A 3, 107) found that the time of setting of thorium phosphate gels increases as increasing quantities of non-electrolytes are added to the gel-forming mixture. They also noticed the peculiar effect of pyridine in

presence of which no gels are obtained and the gelling substance separates out from the gel-forming mixture.

Fleming (*Z. physikal. Chem.*, 1902, **41**, 427) and Holmes (*J. Phys. Chem.*, 1918, **22**, 516) found that the time of setting of silicic acid gel decreases as the temperature is increased. Fells and Firth (*ibid.*, 1925, **29**, 243), however, did not observe any distinct variation in the time of setting of the silicic acid gels on increasing the temperature from 0° to 45°. Bunce (*J. Phys. Chem.*, 1914, **18**, 269) observed that a slight increase in temperature accelerates the gelation of mercuric oxide gels. Szegvari and Schalek (*Kolloid Z.*, 1923, **32**, 318; *ibid.*, **33**, 326) observed that the time of setting of iron oxide gels decreases as the temperature is increased. Prakash (*J. Indian Chem. Soc.*, 1932, **9**, 193) found that the time of setting of all the gels prepared by him decreases as the temperature is raised, excepting in the case of the gels of thorium arsenate, vanadium pentoxide and mercury sulphosalicylic acid, which take a longer time to set at higher temperature and above a certain temperature they do not set at all. Parmar, Mehta and Prasad (*loc. cit.*) found that the time of setting of thorium phosphate gels decreases as the temperature is increased. Hurd, Letteron and Miller (*J. Phys. Chem.*, 1932, **36**, 604, 2194) studied the effect of temperature on the time of setting of silicic acid gels and found that the graphs obtained on plotting $\log t$ against $1/T$ are straight lines. They calculated the heat of activation during the formation of silicic acid gels by means of Arrhenius' equation.

EXPERIMENTAL.

The non-electrolytes used were methyl, ethyl, and propyl alcohols, glycerine and pyridine.

Different amounts of the non-electrolytes were fixed with definite amounts of the solutions of either of the constituents of the gel-forming mixtures and the requisite amount of distilled water was added to make up the total volume of the gel-forming mixture to 10 c. c. The same solutions and the method of mixing were used in the preparation of each gel as described in the paper referred to above and the time of setting was determined in the same manner.

For the purpose of studying the effect of temperature on the setting of the gels, the solution of the constituents of the gel-forming mixtures were taken in two different test tubes and were kept for half an hour in the thermostat maintained at a constant temperature before mixing. The time of setting was then determined at that temperature.

Curves (not shown in the paper) were drawn for the time of setting against the concentration of either of the constituents for several mixtures at different temperatures and the times of setting for the same mixtures at different temperatures were read out from them. At least three such readings were obtained in the case of all gels. The logarithms of the time of setting were then plotted against the reciprocal of absolute temperatures and the slope of the straight lines multiplied by $2.303R$ gave the heat of activation. Owing to the narrow limits of the concentrations of the gel-forming mixture, the heat of activation could not be calculated for the gels of manganese arsenate, zinc arsenate and ceric phosphate.

In the following tables of results, Q represents c. c. of the non-electrolyte and X , c.c. of the constituent containing the anion in the gel-forming mixture. In the tables time of setting has been expressed in minutes unless otherwise stated.

(A) *Thorium arsenate gels.*

Thorium nitrate solution = 6%. Arsenic acid solution = 8.5% .

TABLE I. •

Effect of non-electrolytes.

Thorium nitrate = 5.0 c. c. Arsenic acid = 0.6 c. c.

Time of set for Q in minutes.

	0.00.	0.01.	0.02.	0.20	0.50	1.00.	1.20.	1.50.
Methyl alcohol	10	—	—	—	14	29	50	72
Ethyl alcohol	10	—	—	—	12	24	40	72
Propyl alcohol	10	—	—	—	12	18	28	—
Glycerine	10	—	—	14	25	180	—	—
Pyridine	10	—	30	12 hrs. •	—	—	—	—

TABLE II. •

Effect of temperature.

Thorium nitrate = 6.0 c. c.

Time of set for X in minutes.

Temp.	0.80.	0.70.	0.66.	0.60.	0.56.	0.50.	0.40.
30°	1	3	—	8	11	24	12 hrs
35	4	8	10	28	60	—	—
40	10	30	60	—	—	—	—

TABLE III.

Effect of temperature.

Thorium nitrate = 8.0 c.c.

Time of set for X in minutes.

Temp	0.90.	0.80.	0.76.	0.70.	0.60	0.50.
30°	2	4	—	8	15	55
35	5	8	—	15	52	—
40	16	24	48	—	—	—

TABLE IV.

Heat of activation.

Thorium nitrate.	Arsenic acid.	Time of setting in mins at			Slopes.	Heat of activation
		30°.	35°.	40°.		
6.0 c.c.	0.76 c.c.	1.8	5.0	13.0	-7666 deg	-35120 cal.
"	0.74	2.0	6.0	16.0	-8012	-36700
"	0.72	2.8	7.0	20.0	-8250	-37800
8.0 c.c.	0.90	2.0	5.0	16.0	-7750	-35510
"	0.84	3.0	7.0	20.0	-7786	-35670
"	0.80	4.0	9.0	24.0	-7714	-35340

(B) *Thorium phosphate gels.*

Thorium nitrate solution = 6 %. Phosphoric acid solution = 2 N.

TABLE V.

Effect of temperature.

Thorium nitrate = 5.0 c.c.

Thorium nitrate = 7.0 c.c.

Time of set for X in min.

Time of set for X in min.

Temp.	0.80.	0.70.	0.60	0.50.	1.00.	0.90.	0.80.	0.70	0.60.
30°	18	72	180	12 hr.	20	48	124	188	12 hr.
40	7	32	98	6 hr.	11	26	62	94	4 hr.
5		14	48	172 hr.	6	12	28	56	180'

TABLE VI.

Thorium nitrate	Phosphoric acid.	Heat of activation.			Slopes.	Heat of activation.
		Time of setting in min at 30°.	40°.	50°.		
5.0 c.c.	0.83 c.c.	13.0	5.0	2.0	3818 deg.	17490 cals.
"	0.80	18.0	7.0	3.0	3857	17670
"	0.79	21.0	10.0	4.0	3875	17750
7.0 c.c.	1.02	18.0	9.0	5.0	2615	11980
"	1.00	20.0	11.0	6.0	2643	12110
"	0.97	25.0	14.0	7.0	2666	12210
"	0.94	30.0	16.0	8.5	2643	12110

(C) *Thorium molybdate gels.*

Thorium nitrate solution = 6%. Potassium molybdate solution = 10%.

HCl solution = 2N.

TABLE VII.

Effect of non-electrolytes.

Thorium nitrate = 5.0 c.c. Potassium molybdate = 1.0 c.c. HCl = 1.4 c.c.

	Time of set for Q in minutes					
	0.00.	0.50.	1.00.	1.50.	2.00.	2.60.
Methyl alcohol	2	3	6	12	—	—
Ethyl alcohol	2	4	7	10	—	—
Propyl alcohol	2	5	8	8	—	—
Glycerine	2	—	2	2	—	10

TABLE VIII.

Effect of temperature.

Thorium nitrate = 5.0 c.c.

Thorium nitrate = 7.0 c.c.

HCl = 1.4 c.c.

HCl = 1.4 c.c.

Time of set for X in min.

Time of set for X in min.

Temp.	0.80.	0.60.	0.40.	0.30.	1.00.	0.50.	0.40.	0.30.	0.28.
30°	3	4	7	20	3	6	9	24	—
40	1	2	5	13	1	4	6	12	30
50	1	1	4	10	Instantaneous	2	4	9	18

TABLE IX.

Effect of temperature.

Thorium nitrate = 5.0 c.c. HCl = 2.0 c.c.

Time of set for X in minutes.

Temp.	1.00.	0.80.	0.70.	0.50.	0.40.	0.30.	0.26.
30°	32	10	5	8	16	32	60
40	1	2	—	4	6	12	28
50	Inst.	Inst.	Inst.	1	2	5	18

TABLE X.

Effect of temperature.

Thorium nitrate = 5.0 c.c. Potassium molybdate = 1.0 c.c.

Time of set for X in minutes.

Temp.	0.20.	0.60.	1.00.	1.40.	1.80.	2.00
30°	2	6	4	2	8	32
40	2	4	2	1	1	1

TABLE XI.

Heat of activation

HCl = 1.4 c.c.

Thorium nitrate.	Potassium molybdate.	Time of setting in min. at			Slopes.	Heat of activation.
		30°.	40°.	50°.		
5.0 c.c.	0.40 c. c.	7.0	5.0	4.0	1100 deg.	5040 cal.
"	0.38	8.0	6.0	5.0	1085	4970
"	0.36	9.0	7.0	6.0	1083	4961
"	0.34	11.0	8.0	7.0	1125	5153
"	0.32	14.0	10.0	8.0	1077	4797
7.0 c. c.	0.40	9.0	6.0	4.0	1500	6871
"	0.38	10.0	7.0	5.0	1462	6699
"	0.36	14.0	9.0	7.0	1462	6699

(D) *Stannic phosphate gel.*Stannic chloride solution = 175 g./litre (SnO_2 content = 7.16%).

Phosphoric acid solution = 2N.

TABLE XII.

Effect of non-electrolytes.

Stannic chloride = 3.0 c.c. Phosphoric acid = 1.0 c.c.

Time of set for Q in minutes.

	0.00.	0.10.	1.00.	2.00.	3.00.	4.00.	5.00.
Methyl alcohol	4	—	7	18	36	92	—
Ethyl alcohol	4	—	7	12	21	44	90
Propyl alcohol	4	—	6	8.	11	16.	28
Glycerine	4	—	8	20	46	125	—
Pyridine	4	2	Ppt.	—	—	—	—

TABLE XIII.

Effect of temperature.

Stannic chloride = 3 c.c.

Time of set for X in minutes.

Temp.	1.00.	0.70.	0.60.	0.50.	0.40.	0.30.
30°	4	14	26	60	185	12 hr.
35	3	8	15	26	70	4 "
40	2	5	10	18	36	2 "

TABLE XIV.

Effect of temperature.

Stannic chloride = 4.0 c.c.

Time of set for X in minutes.

Temp.	1.40.	1.00.	0.80.	0.60.	0.50.
30°	8	22	48	180	12 hr.
35	5	14	30	80	4 hr.
40	3	10	20	50	88

TABLE XV.

Heat of activation.

Stannic chloride.	Phosphoric acid	Time of setting at			Slopes	Heat of activation.
		30°.	35°.	40°.		
3.0 c.c.	0.80 c.c.	0.0	5.0	3.0	4286 deg.	19630 cals
"	0.70	14.0	8.0	5.0	4280	19660
"	0.65	18.0	11.0	7.0	4250	19470
"	0.60	26.0	15.0	10.0	4250	19470
4.0 c.c.	1.40	8.0	3.0	3.0	3000	13740
"	1.20	12.0	8.0	5.0	3600	16490
"	1.05	18.0	12.0	8.0	3500	16040
"	1.00	22.0	14.0	10.0	3500	16040

(E) *Stannic Arsenate Gels.*

Stannic chloride solution = 175 g./litre (SnO_2 content = 7.16%).
 Arsenic acid solution = 8.51%.

TABLE XVI.

Effect of non-electrolytes.

Stannic chloride = 3.0 c.c. Arsenic acid = 1.0 c.c.

Time of set for Q in minutes.

	0.00	0.20.	1.00.	2.00	3.00.	4.00.
Methyl alcohol	4	—	11	28	98	—
Ethyl alcohol	4	—	10	22	60	4 hr.
Propyl alcohol	4	—	8	16	36	112'
Glycerine	4	—	10	26	92	—
Pyridine	4	2	Ppt.	—	—	—

TABLE XVII.

Effect of temperature.

Stannic chloride = 3.0 c.c.
 Time of set for X in min

Stannic chloride = 4.0 c.c.
 Time of set for X in min.

Temp	1.00.	0.70.	0.50.	0.40.	0.30	1.50.	1.20.	1.00.	0.80.	0.60.
30°	4	16	68	4 hr.	12 hr	8	20	36	80	12 hr.
35	3	10	36	112'	4 hr.	6	12	22	48	4 hr.
40	2	6	24	60	180	4	8	16	30	94

TABLE XVIII.

Heat of activation.

Stannic chloride	Arsenic acid.	Time of setting at			Slopes.	Heat of activation.
		30°.	35°.	40°.		
3.0 c.c.	0.74 c.c.	13	8	5	4000 deg.	18330 cals.
"	0.70	16	10	6	4000	18330
"	0.63	23	15	10	4000	18330
"	0.60	32	18	12	4000	18330
4.0 c.c.	1.40	12	8	5	3733	17100
"	1.20	20	12	8	3733	17100
"	1.07	27	18	12	3617	16570
"	1.00	36	22	16	3500	16040

EFFECT OF THE ADDITION OF NON-ELECTROLYTES, ETC. 125

(F) <i>Manganese arsenate gels.</i>	(G) <i>Zinc arsenate gels.</i>	(H) <i>Ceric phosphate gels.</i>
Manganese chloride solution = 10%.	Zinc sulphate solution = 10%.	Cerium nitrate solution = 10%.
Potassium nitrate solution = 18%.	Potassium arsenate solution = 18%.	Potassium phosphate solution = 20%.

TABLE XIX.

Effect of non-electrolytes.

Manganese chloride = 4.0 c.c.				Zinc sulphate = 4.0 c.c.				Cerium nitrate = 5.0 c.c.			
Potassium arsenate = 1.0 c.c.				Potassium arsenate = 0.3 c.c.				Potassium phosphate = 0.8 c.c.			
Time of set for Q in min.				Time of set for Q in min.				Time of set for Q in min.			
Ethyl alcohol	3	1	5 Ppt	—	—	—		44	42	56	72
Glycerine	3	1	1	5	2	3		44	52	60	90

TABLE XX.

Effect of temperature.

Manganese chloride = 4.0 c.c.				Zinc sulphate = 4.0 c.c.				Cerium nitrate = 5.0 c.c.			
Time of set for X in min.				Time of set for X in min.				Time of set for X in min.			
Temp.	1.30.	1.00	0.80	0.60	0.35.	0.300.		1.00.	0.80.	0.70.	
25°	1	5	—	—	—	—		—	—	—	
30	Ppt	2	4	—	2	3		32	44	80	
25	—	—	—	—	—	—		24	36	72	
40	—	—	—	—	1	1		18	32	60	
50	—	Ppt.	Ppt.	Inst.	—	—		—	—	—	

DISCUSSION.

The above results show that the addition of increasing quantities of non-electrolytes increases the time of setting of gels in all cases excepting in the case of manganese arsenate gels in which the time of setting is decreased. These observations are in agreement with those of Prasad and Hattiangadi (*loc. cit.*) who also found that the time of setting of acidic gels of silicic acid is increased by the addition of non-electrolytes. It is difficult to explain the peculiar observation in the case of manganese arsenate gels on the existing ideas. The peculiar effect of pyridine is remarkable and is similar to that observed by Prasad and co-workers.

An increase in temperature decreases the time of setting of all gels except that of thorium arsenate gels which shows an increase. The decrease in the time of set can be explained on the basis of an increase in the agglomeration tendency and a decrease in the hydration tendency of the colloidal particles at higher temperature. The increase in the time of setting of thorium arsenate gel with rise in temperature was also observed by Prakash (*loc. cit.*) who explained it on the increased hydrolysis of thorium arsenate at high temperatures. This explanation may not hold good as at constant temperature an increase in the amount of arsenic acid in the gel-forming mixtures causes a decrease in the time of setting. A curious observation is that the peculiar behaviour noticed in thorium molybdate gel-forming mixtures containing 5.0 c.c. of thorium nitrate solution and 2.0 c.c. of HCl solution, to which increasing quantities of potassium molybdate solution are added, at 30° (*cf.* Prasad and Desai, *loc. cit.*) disappears when the temperature is raised.

The values of the slope given for different gels are not changed appreciably with an increase in the amount of the salt containing the anion of the gelling substance. The difference in the values of heat of activation with a change in quantity of the salt containing the cation of the gelling substance in the mixture is, however, quite appreciable. If these differences are neglected and an average value is taken, it is difficult to conclude that the heat of activation is a characteristic property of a gel, distinct from the other.

DYES DERIVED FROM ACENAPHTHENEQUINONE. PART VII. 2-(5-CHLORO)-THIONAPHTHENE-ACENAPHTHYLENE- INDIGOS.

BY SISIR KUMAR GUHA.

5-Chloro-3-hydroxythionaphthene has been condensed with acenaphthenequinone and a few of its derivatives and also phenanthraquinone. The newly prepared 5-chloro-indigoid vat dyes, described here, are found to be distinctly deeper than the corresponding 5-methyl compounds already studied.

In a series of papers, a systematic and detailed study has been made of the influence of the methyl group on the colour of 2-thionaphthene-acenaphthyleneindigos (Ciba Scarlet G and its derivatives), 3-indole-2'-thionaphtheneindigos (Thioindigo Scarlet R and its derivatives), 2-thionaphthene-1'-aceanthryleneindigo, bis-2-thionaphthene-ethyleneindigo, and benzylidene-2-thionaphthenes, according as it is present in the 4-, 5-, or 6-position of the thionaphthene nucleus of the molecule, i.e., in the *ortho*, *meta*, and *para* position with reference to the carboxyl group (Guha, *J. Indian Chem. Soc.*, 1933, **10**, 679; 1936, **13**, 94; 1938, **15**, 20; Guha and Basu-Mallick, *ibid.*, 1934, **11**, 395; Guha, *ibid.*, 1937, **14**, 240, 1938, **15**, 501; 1935, **12**, 659; 1937, **14**, 709; 1938, **15**, 359; cf. Auwers and Arndt, *Ber.*, 1909, **42**, 541; Friedländer, *Monatsh.*, 1909, **30**, 347).

The colour, dyeing properties and absorption spectra revealed the fact that in each of the series mentioned above, the 5-methyl derivative is the deepest in shade, the second is the parent compound, next comes the 4-methyl compound and last is the 6-methyl dye. From the present state of our knowledge regarding colour and chemical constitution it is difficult to explain why the introduction of the methyl group in the 4- and 6-position should lighten the colour of the parent substances, and deeper shades should be obtained when the same group occupies the 5-position. It was felt that further work is necessary on the subject and the effect of the introduction of other atoms or groups in different positions must be determined before it is possible to put forward any comprehensive generalisation.

With this object in view, it was decided to make a systematic study of the influence of the position of the chlorine atom, precisely in the same manner as has been already done in the case of the methyl group in the acenaphthenequinone, isatin, aceanthrenequinone, conjugated-indigo analogues and thioindigenide series (Guha, *loc. cit.*). For this purpose

5-chloro-3-hydroxythionaphthene (D. R. P. 224567, Friedlander, 10, 474; Auwers and Thies, *Ber.*, 1920, 53, 2285) was condensed with acenaphthenequinone, and its 3-chloro-, 3-bromo-, and 1-methoxy derivatives, and the corresponding indigoid vat dyes obtained.

These newly prepared dyes are soluble in pyridine and nitrobenzene and sparingly soluble in alcohol. The parent compound as well as its chloro and bromo derivatives dissolve in concentrated sulphuric acid giving deep green solutions, but the methoxy derivative, when treated similarly, produces a blue solution. From sulphuric acid solutions, on the addition of water, the dyes are reprecipitated unchanged in a finely divided state, suitable for dyeing on wool from an acid bath. When heated strongly above 312°, they melt and quickly volatilise evolving coloured vapours of the respective substances. The dyeing shades on wool from a dilute sulphuric acid bath and on cotton from an alkaline hydrosulphite vat, develop well, except in the case of the methoxy derivative where full shades are not obtained. A considerable amount of difficulty has been experienced in reducing it in the vat. The violet-red alkaline vat obtained in this case, after repeated trial, turns into pink by air oxidation [*cf.* 2-thionaphthene-8'(1'-methoxy)-acenaphthyleneindigo, its 4-, 5- and 6-methyl derivatives; 2:3-naphthathionaphthene-8'-(1'-methoxy)acenaphthyleneindigo, Staudinger, Goldstein and Schlenker, *Helv. Chim. Acta*, 1921, 4, 342; Guha, *loc. cit.*].

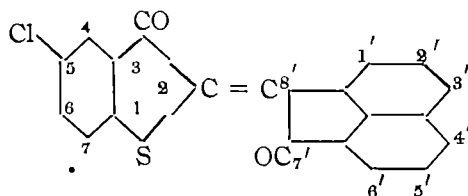
Lastly, another indigoid dye in the phenanthraquinone series 2-(5-chloro)-thionaphthene-9'-phenanthreneindigo was prepared with the object of comparing its dyeing properties with those of 2-(5-chloro)-thionaphtheneacenaphthyleneindigo. The dark chocolate coloured substance dyes wool in brown shades from an acid bath and imparts a faint brown colour to cotton from a yellow alkaline vat.

The 5-chloro dyes, described in this paper, produce on cotton shades which are distinctly deeper than those obtained from the corresponding 5-methyl compounds (Guha, Guha and Basu-Mallick, *loc. cit.*). A comparative statement of the shades from some of them is tabulated below.

A = Acenaphthylene-indigo. T = Thionaphthene.

Compounds	Shades on cotton.
2-(5-Chloro)-T-A	Dark red
2-(5-Me)-thionaphthene-A.	Scarlet red
2-(5-Chloro)-T-8' (3'-chloro) A.	Dark red.
2-(5-Me)-T-8' (3'-chloro)-A.	Scarlet red.
2-(5-Chloro)-T-8' (3'-bromo)-A.	Dark red.
2-(5-Me)-T-8' (3'-bromo)-A.	Scarlet red.
2-(5-Chloro)-T-8' (1'-methoxy)-A.	Pink
2-(5-Me)-T-8' (1'-methoxy)-A.	Pink.

E X P E R I M E N T A L .

2-(5-Chloro)-thionaphthene-acenaphthyleneindigo.

It separated as light violet-red well-defined needles from a solution of acenaphthenequinone (0.364 g.) and 5-chloro-3-hydroxythionaphthene (0.368 g.) in glacial acetic acid (70 c.c.) and 4 c.c. of concentrated hydrochloric acid on boiling the mixture for 15 minutes. The dye (0.492 g.) crystallised from xylene in foliated hair-like dark red needles. It is soluble in xylene, difficultly soluble in amyl alcohol, moderately soluble in chloroform and benzene; sparingly soluble in acetic acid. The dyeing shade on wool is dark red from an acid bath; cotton is also dyed in the same colour from an alkaline deep violet vat. (Found: Cl, 10.48. $C_{20}H_9O_2ClS$ requires Cl 10.18 per cent)

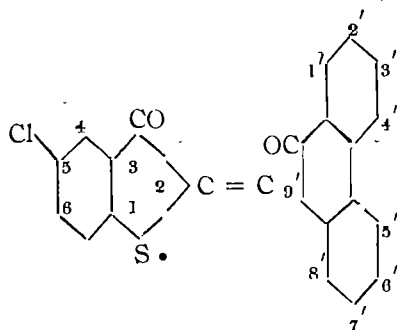
2-(5-Chloro)-thionaphthene-8'-(3'-chloro)-acenaphthyleneindigo was obtained by heating 3-chloroacenaphthenequinone (0.433 g.) and 5-chloro-3-hydroxythionaphthene (0.369 g.) in 55 c.c. of glacial acetic acid and 6 c.c. of concentrated hydrochloric acid for 15 minutes, as fine red needles which when dried become brownish red. The product (0.451 g.) was crystallised from pyridine when it came down in the form of long needles. It dyes wool in brownish red shades from an acid bath and cotton in dark red shades from an alkaline deep violet-blue vat. It resembles the parent compound in solubility. (Found: Cl, 18.78. $C_{20}H_8O_2Cl_2S$ requires Cl, 18.56 per cent).

2-(5-Chloro)-thionaphthene-8'-(3'-bromo)-acenaphthyleneindigo was prepared in the same way as the preceding compound from 3-bromoacenaphthenequinone (0.398 g.) and 5-chloro-3-hydroxythionaphthene (0.282 g.) in 35 c.c. of glacial acetic acid and 3 c.c. of concentrated hydrochloric acid. The red crystalline precipitate which on drying became brownish red (0.422 g.) was crystallised from pyridine. It formed thread like needles. It resembles the chloro compound in other properties. (Found: S, 7.29. $C_{20}H_8O_2BrClS$ requires S, 7.48 per cent).

2-(5-Chloro)-thionaphthene-8'-(1'-methoxy)-acenaphthyleneindigo separated from β -methoxyacenaphthenequinone (0.636 g.) and 5-chloro-3-hydroxythionaphthene (0.5535 g.) in 100 c.c. of glacial acetic acid and 5 c.c.

of concentrated hydrochloric acid when boiled for 25-30 minutes as a dark red crystalline precipitate. The dye (0.283 g.) when crystallised from pyridine formed needle-shaped crystals. It is difficultly soluble in xylene, chloroform, moderately soluble in acetic acid. It dyes wool in light red shades. (Found : Cl, 9.74. $C_{21}H_{11}O_3$ Cl S requires Cl, 9.37 per cent).

2-(5-Chloro)-thionaphthene-9'-phenanthreneindigo.



The hot solution of phenanthraquinone (0.624 g.) and 5-chloro-3-hydroxythionaphthene (0.5535 g.) in glacial acetic acid (30 c.c.) was treated with 4 c.c. of concentrated hydrochloric acid and shaken, when a crystalline mass separated. Glacial acetic acid (20 c.c.) was added and the solution boiled for 20 minutes. The dye (0.264 g.) was collected, washed with acetic acid, hot water and crystallised from pyridine in dark chocolate needles. It is soluble in pyridine, nitrobenzene, aniline; difficultly soluble in xylene, sparingly soluble in toluene, acetic acid and alcohol. (Found : Cl, 9.71. $C_{22}H_{11}O_2ClS$ requires Cl, 9.47 per cent)

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Received January 28, 1939.

MANGANESE CONTENT OF INDIAN FOODSTUFFS AND OTHER MATERIALS.

BY M. N. RUDRA.

Manganese contents of a number of vegetable and animal materials have been determined. Rohit fish scales have been found to be richest in manganese content (8.831 mg./100 g. dry) and tender amaranth leaves are richest among vegetable materials. Milk is poor in manganese content.

The importance of manganese in plant nutrition is now recognised. Manganese appears also to be important in animal nutrition. Within certain concentrations manganese appears to stimulate tissue respiration and formation of autotoxin in animals (*cf.* Oettingen, *Physiol. Rev.*, 1935, **15**, 175). Kemmerer, Elvejem and Hart (*J. Biol. Chem.*, 1931, **92**, 623) found that the addition of traces of manganese to a milk diet had a favourable effect upon the growth of mice. Normal ovulation failed in mice grown on milk supplemented with iron and copper only but the addition of manganese produced normal oestrous cycles. Orent and McCollum (*J. Biol. Chem.*, 1931, **92**, 651) found that on manganese-free diets young rats grow normally, females have normal oestrous cycles and bear normal number of youngs but they failed to suckle the youngs. In male rats complete degeneration of the testis occurred with consequent sterility. Keil, Keil and Nelson, (*Amer. J. Physiol.*, 1934, **108**, 215), however, obtained reproduction in the first generation and no reproduction in the second generation of rats on a manganese-free diet.

Manganese thus appears to be an important mineral in animal nutrition and Everson and Daniels (*J. Nutrition*, 1934, **8**, 497) suggest a daily dose of 0.2—0.3 mg. of manganese per kg. of body weight in the case of children. The importance of the knowledge of the distribution of manganese in foodstuffs is, therefore, apparent. No attempt appears to have yet been made to find out manganese distribution in our foodstuffs, although in Europe and America such work has been carried out (*cf.* Oettingen, *loc. cit.*). Boyd and De (*Indian. J. Med. Res.*, 1933, **21**, 109) carried out some spectrographic analysis of foodstuffs and found manganese lines but no quantitative data were given. In the present study the manganese content of a number of Indian foodstuffs and some other materials has been determined. The determinations were made by the method described by Skinner and Peterson (*J. Biol. Chem.*, 1930, **88**, 347) with modifications when found

necessary. Two of the items were, in addition, determined by the method of Richards (*Analyst*, 1930, 65, 554). The results are given in the following tables.

TABLE I.
Manganese content of plant materials.

Name.	Scientific name.	Mn content mg./100 g. (dry basis).
Cereals		
Rice, sun-dried, polished	<i>Oryza sativa</i>	1.34
„ parboiled	„	1.06
Wheat	<i>Triticum vulgare</i>	2.07
Pulses		
Green gram	<i>Phaseolus mungo</i>	1.93
Bengal gram	<i>Cicer arketinum</i>	3.00
Vegetables, leafy		
Cabbage	<i>Brassica oleracea capitata</i>	5.69
Amaranthus, tender	<i>Amaranthus gangetica</i>	6.37
„ mature	„	5.86
Vegetables, root		
Carrot	<i>Daucus carota</i>	0.754
Khol khol	<i>Brassica oleracea caulorapa</i>	5.21
Vegetables, other		
Cauliflower	<i>Brassica oleracea botrytes</i>	2.62
Brinjal	<i>Solanum melongena</i>	2.40
Fruits		
Guava	<i>Psidium guajava</i>	1.59
Chillies, green	<i>Capsicum annum</i>	1.64
Jujuba plum	<i>Zizyphus jujube</i>	0.982
Indian gooseberry	<i>Emblica officinalis</i>	2.90

TABLE II.

Manganese content of animal materials.

Name.	Scientific name	Manganese content mg./100 g. moist tissue.	dry tissue
Goat			
Liver		0'338	1'233
Muscle		0'0170	0'073
Blood, whole		0'010*	—
Blood, serum		0'004*	—
Milk		0'0075*	—
Milk, cow's		0'006*	—
Rohit fish, muscle	<i>Labeo rohita</i>	0'0455	'248
Do scale	„	—	8'831
Calbosh fish, muscle	<i>Labeo calbosh</i>	0'0442	0'243
Mrigal fish „	<i>Cirrhina mrigala</i>	0'0384	'201
Catla fish „	<i>Catla catla</i>	0'0382	0'211
Chital fish „	<i>Notopterus chitala</i>	0'0502	0'241

DISCUSSION.

From the above results it will be seen that of the materials investigated amaranthus (*nate sag*) among plant materials and scales of rohit fish among animal materials are richest in manganese content. It is also significant that plant products, which are rich in manganese content, are also generally rich in vitamin C content. Thus amaranth, *brassica oleraceas*, chillies etc., which are rich in vitamin C content are also more or less rich in manganese contents. Indian gooseberry is very rich in vitamin C content and its manganese content is also high. Of the two root vegetables, knol khol and carrot, the former is rich and the latter is very poor in vitamin C content. Their manganese contents are also similarly high and poor, namely 5'21 and 0'754 mg./100 g. respectively. That manganese content of living materials runs almost parallel with their vitamin C content is brought out more clearly from the following table

* per 100 c.c.

which gives the manganese content and vitamin C content (determined titrimetrically with indophenol reagent) of tender and mature amaranth leaves.

TABLE III.

Manganese and vitamin contents of amaranth leaves.

	Mn content mg./100 g. dry.	Vit. C content mg /100 g moist
Tender	6.37	151.0
Mature	5.86	140.0

The author holds the opinion that manganese plays an important rôle in the biological synthesis of vitamin C. It has been found that manganese has considerable influence in the vitamin C content of seedlings and the hypothesis has been advanced that manganese is an indispensable link in the mechanism by which plants synthesise vitamin C initially (communicated to the press ; cf. also *Nature*, 1938, **141**, 203).

The high manganese content of Rohit fish scales (and probably of other fish scales) should encourage one to investigate the possibility of utilising them in the manufacture of edibles, jelly crystals for example.

I wish to thank Prof. T. N. Seth for giving facilities.

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Received March 4, 1939

CHEMICAL EXAMINATION OF THE WAX FROM SUGARCANE.

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Sugarcane wax extracted from the press-mud of a sulphitation factory has been found to contain 43.7% of acids and 53.0% of non-saponifiable material. By the usual method of ester fractionation under a reduced pressure, the component acids have been found to be resin acid (4.5%), caproic acid (0.6%), palmitic acid (22.7%), stearic acid (22.4%), oleic acid (41.5%) and arachidic acid (3.3%). The non-saponifiable material has been resolved into primary alcohols, secondary alcohols, and paraffin fractions by treating it with phthalic anhydride. The non-saponifiables consist of about 80% primary alcohol *n*-triacontanol or myricyl alcohol, about 10% is a mixture of sterols, from which brassica, stigma and sitosteriols have been isolated by the fractional crystallisation of their bromination products and about 5% of an aliphatic paraffin, *n*-pentatriacontane ($C_{35}H_{72}$). The wax does not contain any dibasic acid or oxy-acid.

Sugar cane wax was observed by Avequin (*Ann. chim. phys.*, 1840, *ii*, 75, 214) to be a mixture of different bodies, occurring on the exterior of cane. In some varieties it may amount to about 0.05% on cane and in some it might be nil. When the cane is crushed the major portion of this wax goes along with the juice. Its presence in the juice is as much detrimental to the manufacture of sugar as of any other non-sugars. During the process of clarification the wax is precipitated and goes along with the press-cake. The chemical nature of the wax extracted from press-cake has been studied by various investigators. There have been two reasons to initiate this investigation; the first to eliminate it as completely as possible from the clarified juice and the second to utilise it as a commercial commodity.

Wijnberg (*Deuts. Zuckerind.*, 1909, **34**, 629; *J. Soc. Chem. Ind.*, 1909, **28**, 991) studied the composition of cane-wax and also patented the process for its recovery from the press-cake for industrial use (B.P. 25669 of Nov., 1910). He found that 70% of the wax consist of the glycerides of oleic, linolic, palmitic and stearic acids along with hydroxy-acids, resin acids, phytosterols and colouring matters and the remaining 30% contain 45% of myricyl alcohol, 35% of a non-primary crystalline alcohol and at least one more crystalline substance, poor in oxygen (m.p. 88-90°) corresponding to $C_{33}H_{66}O$.

Bosz (*Arch. Suikerind in Nederlandsch-Indie*, 1920, **28**, No. 25, 974) examined the wax, extracted from the press-cake of a sugar factory in Java. He could not get any positive indication towards the presence of sterols when he applied the ordinary colour reaction to the wax. He

identified stearic, palmitic, caproic and formic acids along with myricyl alcohol. He does not mention any unsaturated compound in the wax.

While the present work was in progress Tetsuo Mitsui (*J. Agric. Chem. Soc. Japan*, 1937, **18**, 494) obtained phytosterols from the sugarcane wax and also suggested that it could be utilised as a starting material for the preparation of hormones. He has not mentioned much about the nature of the phytosterols.

Recently a good deal of work has been done on the plant and insect waxes and a number of ketones, ketonic alcohols and paraffins have been isolated. Cane-wax as well might contain some such compounds which might have escaped the observation of the previous workers. It was considered desirable to re-examine the wax and identify the individual components with the help of the more recent methods adopted for the study of other fats and waxes. The composition of the component acids has been determined by the usual ester fractionation method of Hilditch *et al.* (*J. Soc. Chem. Ind.*, 1927, **46**, 1721) and the non-saponifiable constituents have been studied by phthalic anhydride method of Chibnal and Piper (*Biochem. J.*, 1931, **25**, 2095).

EXPERIMENTAL.

In March 1937, fully riped cane (Co. 213) was crushed in the Sri Ram Sugar factory, Ltd., Bobbili.* The juice was clarified by the double sulphitation process, when a clear juice practically free from wax was obtained. The scum was maintained at 7° R. W. and kieselguhr was added to aid the filtration. The cake, so obtained, was dried up at 100°, powdered and extracted with benzene which was found in a preliminary experiment to be the most suitable solvent. The wax was dark green in colour and its physical and chemical constants are compared with those observed by the other workers.

TABLE I.

	Wijnberg.	Lewkowitsch.	Bosz	Present workers.
Sap. value	167.9	81.2	177.0	133.5
Iodine "	60.0	87.7	—	31.5
Acid "	38.6	11.9	47.3	23.4
Acetyl "	55.6	—	—	89.6
Non-saponifiables	58.8	69.1	—	43.7
Melting points	55.79°	58.59°	60-62°	68.7°

* We express our gratitude to the mill authorities for allowing us this facility.

The Component Acids of the Wax.

The crude wax (250 g.) was purified by boiling successively with 5% solution of sodium sulphite, 3% solution of hydrochloric acid and finally a number of times with distilled water till free from the mineral acids and other inorganic matters. It was dried at 90° under reduced pressure. The dry wax (200 g.) was dissolved in benzene (1000 c.c.) and saponified by boiling with an excess of 5N alcoholic caustic soda. After distilling off the solvent the soap was mixed with silver sand and fried till it was friable. The non-saponifiables were extracted from the soap with acetone and petroleum ether consecutively but as the soap was found to contain some non-saponifiables, it was converted into calcium soap and extracted with a little dry benzene. The small quantity of soap extracted with the non-saponifiables was recovered and added to the main bulk of soap by washing the ether solution of the non-saponifiables with water. Acids were liberated from the soap by boiling with glacial acetic acid, 106 g. of the acids (sap. equiv., 294; I.V., 39.5) and 87.5 g. of non-saponifiables (I. V., 10.5) were obtained. The rest were water-soluble and steam-volatile materials.

Separation of Solid and Liquid Acids.

The solid and liquid acids were separated by the Twitchell's method (*Ind. Eng. Chem.*, 1921, **13**, 806) with a slight modification of recrystallising the sticky alcohol-insoluble lead salts from ether.

Fractions obtained from 30 g. of acids (sap. equiv., 294; I. V., 39.5) are given in Table II.

TABLE II

	Fractions.	Quantity	Sap. Equiv.	I. V.
A	From the lead salts, soluble in alcohol and ether	12.2 g.	279	80.2
B	From the lead salts, soluble in ether	8.5	302	18.9
S	From the lead salts, insoluble in ether and alcohol	9.2	314	14.8

In order to separate any dibasic or oxy-acid, all these fractions were treated separately with light boiling petroleum ether, but no insoluble matter was obtained. This proves the absence of dibasic and oxy-acids.

The acid from A (2 g.) was oxidised with potassium permanganate (2 g.) in alkaline solution at about 60°, according to the method of Lapworth

and Mottram (*J. Chem. Soc.*, 1925, **127**, 1678). The saturated acid, extracted from the product of oxidation by petroleum ether, was identified to be palmitic acid, m.p. 61° , unchanged when mixed with an authentic sample. In the oxidation product no tetrahydroxy nor any hexahydroxy acid could be found; only dihydroxystearic acid, m.p. 129° (either alone or mixed with an authentic sample of Δ^9 -10-dihydroxy acid) was detected. The only unsaturated fatty acid in this wax is oleic acid.

From the acid fraction (E) palmitic, stearic and oleic acids were identified.

The acids identified from (S) were oleic and stearic acids along with archidic acid, m.p. 75° and equiv., 307.5 (calc. for $C_{20}H_{40}O_2$ Equiv., 312).

The Estimation of Resin Acids.

Twitchell's method (*J. Soc. Chem. Ind.*, 1891, **10**, 804) was adopted for the estimation of the resin acids and their separation from the fatty acids. A solution of mixed acids (65 g.) in absolute alcohol (800 c.c.) was saturated with hydrochloric acid gas and allowed to stand for 3 hours at the room temperature and then boiled with distilled water till an oily layer was obtained. This oily layer was taken up in ether, and washed free from hydrochloric acid. An aliquot part of this ethereal solution was taken up with an equal volume of neutral alcohol and the amount of resin acid (4.5% on the total acids) which remained unesterified, was calculated by titrating it against a $N/2$ -solution of caustic potash. The rest of the ethyl ester of fatty acids was washed neutral with sodium carbonate solution and water and fractionated.

Fractional Distillation of the Ethyl Esters.

50 G. of neutral ethyl esters of the fatty acids (sap. equiv., 306.0 and I. V., 37.5) were fractionated from a packed column Willstatter's bulb under a pressure of 0.2 mm. The results are given in Table III.

TABLE III.

Fraction.	B. p.	Wt	Sap. Equiv.	I. V.	Acids identified.
1	110-130°	9.0 g.	282.2	38.0	Palmitic and oleic.
2.	132°	23.5	298.8	49.5	Palmitic, stearic and oleic
3.	168°	3.0	310.3	62.8	"
4.	170°	3.0	319.5	38.4	Stearic, archidic and oleic.
5.	Residue	10.4	332.0	6.5	Stearic and archidic.

* The residue contained 3.02% of non-saponifiable materials of corrected values of sap. equiv., 322.0 and I. V., 6.7.

Identification of Caproic Acid.

The boiling point and the equivalent of the fraction 1 were too low for the ethyl esters of a hexadecenoic acid. No acid lower than palmitic could be crystallised out. Some lower and steam-volatile acid must be in this wax. A good lot of it might have escaped at the time of the liberation of the acids. Therefore, 80 g. of the crude wax were saponified and the soap was splitted up by the addition of an excess of dilute sulphuric acid. It was distilled in steam till no more acid was coming in the distillate and 3 litres of distillate were collected. An excess of sodium hydroxide was added and the volume was boiled down to 400 c.c. The steam-volatile non-saponifiable materials such as myricyl alcohol and a little of essential oil, smelling like geraniol, were extracted with ether and the soap was splitted up by adding dilute hydrochloric acid, 0.63 g. of acid being obtained (0.1126 g. of the Ba salt gave 0.0421 g. of Ba. Found: M.W., 115.05. Calc. for $C_6H_{12}O_2$: M.W., 116.0). The distillate did not respond to any test for formic acid.

Composition of the Acidic Component of the Wax.

TABLE IV.

Acids.	Wt. of fatty acids	Wt. of total acids.	% on the wax.
Caproic	0.7%	0.6%	0.3
Palmitic	29.0	27.7	14.7
Stearic	23.4	22.4	11.8
Oleic	43.5	41.5	22.0
Archidic	3.4	3.3	1.8
Resinous acid	...	4.5	2.4

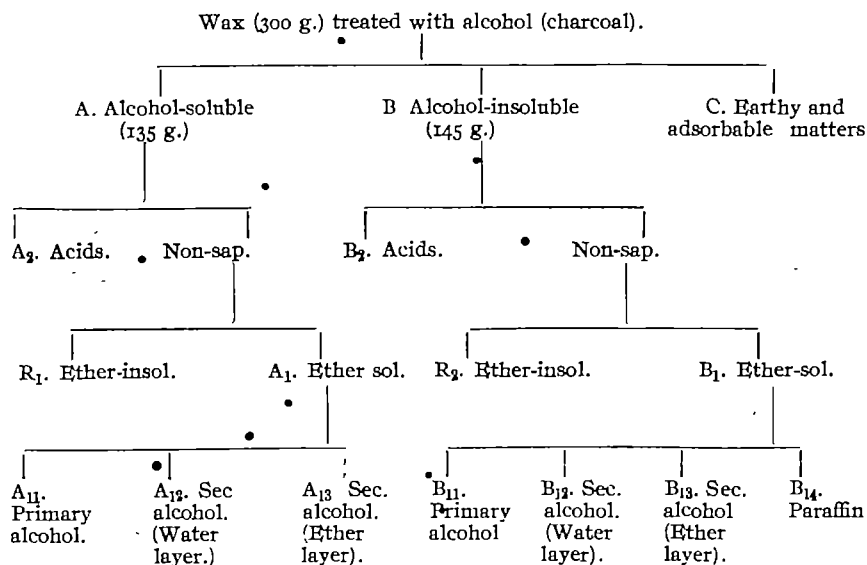
Disruptive Oxidation of the Unsaturated Acid

15 G. of the ethyl ester (sap. equiv., 310.5 and I.V., 81.5), obtained by the fractional crystallisation of the lead salts of the acids of fractions 1 and 2 (Table III) were oxidised by potassium permanganate in acetone solution according to the methods of Armstrong and Hilditch (*J. Soc. Chem. Ind.*, 1925, **44**, 447). In the product of oxidation azelaic acid (m.p. and mixed m.p. 105°) and nonolic acid (equiv., 160) were obtained.

The Non-saponifiables of the Wax

The crude dark coloured wax (300 g.) was resolved into two fractions by boiling thrice, with two litres of 25 % alcohol and filtering the solution hot each time. As some of the non-saponifiables were steam-volatile, the method of purification adopted in the acidic portion had to be given up. The alcohol-soluble portion had in it most of the green colour of the wax which was decolourised by boiling for 1 hour with animal charcoal in alcohol solution and filtering hot; 135 g. of clear white wax were obtained. The residue was dissolved in benzene and decolourised with animal charcoal, when 145 g. of light brown wax were obtained. Both these fractions were examined separately. The general scheme of separation is given in the following table.

TABLE V.



Wax A.—The alcohol-soluble wax fraction A (125 g.) was dissolved in alcohol (1500 c.c.) and saponified by boiling with alcoholic caustic potash (12%, 1200 c.c.) for 8 hours. After complete saponification the potassium soap was converted into calcium soap by adding 2 litres of 8% solution of calcium chloride in alcohol and boiling for another 2 hours. It was filtered hot. The non-saponifiable materials were separated from the soap in the

filtrate. As the calcium soap still contained some non-saponifiables, it was extracted successively with alcohol, acetone and benzene. The residues of all these extracts were added to the main filtrate, which was again saponified with an excess of alcoholic caustic potash. All the calcium soap was converted into potassium soap and the precipitate of calcium hydroxide was filtered off. The solvent was evaporated off from the filtrate. The residue was taken up in ether and washed with water in order to make it free from soap, glycerine and other water-soluble matters. The portion (R_1), least soluble in ether, was crystallised out and identified to be myricyl alcohol. The residue A from the mother-liquor was subjected to the phthalic anhydride treatment.

Fraction A_1 .—The fraction A_1 (20 g.) was mixed with phthalic anhydride (20 g.), pyridine (8 c.c.) and refluxed at a temperature of 125° for 18 hours. The resulting mass was poured while hot into a dilute solution of hydrochloric acid to remove pyridine. The black solid layer, which was obtained on cooling, was boiled a number of times with water to remove the excess of phthalic anhydride. It was then dissolved in ether (600 c.c.) and the ethereal solution was treated with an excess of slightly warm aqueous solution of sodium carbonate (4 %). A grey precipitate of the sodium phthalate of primary alcohol was thrown down, which could not be separated easily. The whole mixture was separated by centrifuging into three separate layers, viz., ether layer, aqueous layer and the solid layer. The solid and aqueous layers were extracted again with ether and the extracts were added to the main ether solution. Solvent was evaporated off from this ether solution and the residue was taken up in alcohol. All these three fractions were saponified with 20 % alcoholic caustic potash for 18 hours and the non-saponifiables A_{11} , A_{12} and A_{13} were extracted from the soaps in usual manner.

The Fraction A_{11} .—This fraction contained the primary alcohol which after crystallisation from benzene-alcohol mixture (1:1) melted at 85° . It has been further identified along with the other primary alcoholic portion of the wax and found to be myricyl alcohol. From the mother-liquor a slimy yellow liquid was obtained, whose quantity was too small for identification.

Fraction A_{12} .—The sodium phthalate of this portion was soluble in water. It was crystallised from benzene-alcohol mixture (1:1) and acetone-methyl alcohol mixture (1:3) when it melted at $133-134^\circ$ (not sharp). It gave all colour tests (cf. Liebermann-Burchard, *Chem. Zentr.*, 1890, I, 25; Salkowski, *Z. physiol.*, 1908, 57, 523; Rosenheim, *Biochem. J.*, 1927, 23, 47) for sterols and hence it was a mixture of sterols.

Fraction A₁₃.—It was crystallised from acetone and methyl alcohol mixture (1 : 3) when it melted at 135°, m. p. rising up to 137·5° after two crystallisations from the same solvent. Just like A₁₂, it gave all the tests for sterols.

Wax B (Alcohol-insoluble).—The alcohol-insoluble wax B (120 g.) was saponified and the non-saponifiable was separated in the same way as A, excepting that benzene, instead of alcohol was used for dissolving the wax. From this fraction the portion R₂, least soluble in ether, was separated out and it was identified to be myricyl alcohol.

Fraction B₁.—The ether-soluble non-saponifiable from B was separated into primary alcohol, secondary alcohol and paraffin by treating with phthalic anhydride. In this case four fractions were obtained. The last fraction B₁₄ separated as an alcohol-insoluble substance when the sodium phthalate of the secondary alcohol was dissolved in alcohol. According to Chibnal *et. al.* (*loc. cit.*) it could be a paraffin or a ketone.

Fraction B₁₁.—This fraction on crystallisation from benzene-alcohol mixture was found to be myricyl alcohol.

Fractions B₁₂ and B₁₃.—Just like the fractions A₁₂ and A₁₃ they were found to be a mixture of sterols.

Fraction B₁₄.—This fraction was treated with phthalic anhydride again. After the elimination of the small quantity of primary and secondary alcohols, it was found to be a light powder, m.p. 74-75° (shrinking at 73°). It did not respond to any test for a ketone. It was inactive towards most of the reagents. This is a characteristic property of higher aliphatic paraffins (*cf.* Piper, Chibnal, Hopkins and Smith, *Biochem. J.*, 1931, **25**, 2072) which have a transition point as well as a melting point. [Found: C, 85·3; H, 14·3; M. W. (cryoscopic), 498. Calc. for C₃₅H₇₂: C, 85·44; H, 14·66 per cent. M.W., 492). All the properties agree with those for *n*-pentatriacontane.

Myricyl Alcohol.—The fractions R₁, R₂, A₁₁ and B₁₁ were found to be similar, melting at 85-86° and there was no depression when the crystallised product of any of them was mixed with the other. They were mixed up and the mixture was converted into an acetate (m.p. 75-76°) and a benzoate (m. p. 69-70°). By fusing it with soda lime at 250° melissic acid (C₃₀H₆₀O₂, m.p. 91°) was obtained. The acetate of this alcohol had the saponification equivalent of 482. (Calc. for myricyl acetate C₃₂H₆₄O₂: 480). It is obvious that this primary alcohol is *n*-triacontanol or myricyl alcohol (C₃₀H₆₂O).

Individual Sterols from the Mixture.—The fractions A₁₂, A₁₃, B₁₂ and B₁₃ were found to be similar. No pure product could be identified from

any of them. All these fractions were mixed up and the mixture had an optical rotation (in chloroform solution) $[\alpha]_D^{28}, -39.8^\circ$. It was converted into an acetate by boiling with acetic anhydride and 3 g. of this acetate were dissolved in ether (30 c. c.) and brominated according to the method of Windaus and Hauth (*Ber.*, 1906, **39**, 4378). The first crop of crystals was recrystallised from chloroform-alcohol mixture (1:1), when shining rhombic crystals were obtained. It decomposed at 205° . These crystals when debrominated gave crystals of the acetate as six-sided leaflets, m.p. $152-53^\circ$. There was no depression in the m.p. when it was mixed with an authentic sample of brassica sterol acetate obtained from mustard seed oil.

The mother-liquor was concentrated and left to crystallise at a temperature of 3° . Some more hexagonal crystals were obtained, which on recrystallisation were found to decompose at 203° . Probably it contained a small quantity of tetrabromo-brassica sterol acetate as well. On debromination the acetate of this sterol was obtained, m.p. 140° . The sterol obtained by the saponification of the acetate was needles, m.p. $168-69^\circ$. It had got all the characteristics of stigmasterol.

From the mother-liquor, after separation of the two crops of crystals of the bromoacetates of these two sterols, the solvent was evaporated off. The residue was debrominated. The acetate, when crystallised from absolute alcohol, melted at 134° and the sitosterol obtained on saponification melted at 143° .

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Received December 19, 1938.

CONSTITUTION OF HALOGENATED RESACETO AND PROPIOPHENONES.

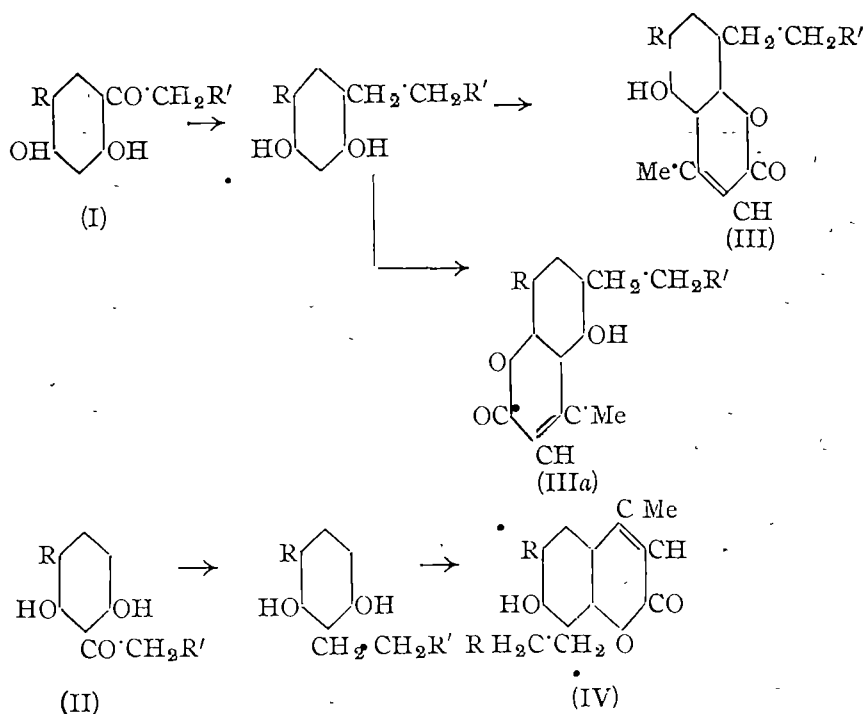
BY DUHKHAHARAN CHAKRAVARTI AND NIRANJAN CHAKRAVARTY.

4-Chlororesorcin when heated with acetic acid and propionic acid, yields 5-chloro-resacetophenone and 5-chlororespropiophenone respectively. Similarly 4-bromoresorcin yields with acetic acid 5-bromoresacetophenone. The constitution of these halogenated ketones has been determined and it has been found that they do not condense with acetoacetic ester to form coumarins.

The work described in this paper, is the outcome of the investigations carried to establish the constitution of the coumarins obtained by Agarwal and Dutt (*J. Indian Chem. Soc.*, 1937, **14**, 109) by the condensation of resacetophenone with acetoacetic ester and its alkyl derivatives. It may be noted in passing that the coumarin, described by Agarwal and Dutt (*loc. cit.*), is not a coumarin at all but pure resacetophenone and it is not possible to effect the condensation of resacetophenone with acetoacetic ester under the experimental conditions of the authors (*cf.* Chakravarti and Chakravarty, *Science and Culture*, 1937, **3**, 244; Sethna, Shah and Shah, *Current Science*, 1937, **6**, 93; Limaye, *Rasayanam*, 1, 101).

Halogenated resaceto- and propiophenones have been prepared from the corresponding halogenated resorcins by Nencki's reaction. 4-Chlororesorcin and 4-bromoresorcin (Chakravarti and Ghosh, *J. Indian Chem. Soc.*, 1935, **12**, 791; Chakravarti and Mukerjee, *ibid.*, 1938, **15**, 493) have been found to yield halogenated ketones readily, which might have the alternative structures (I) or (II). In order to decide between the two structures the halogenated ketones have been reduced by Clemmensen's method and the halogenated C-alkyl-resorcins, thus obtained, have been condensed with acetoacetic ester producing coumarins. If the halogenated ketones possess the structure (I) it would produce a 5-hydroxycoumarin (III or IIIa) but if it has the constitution (II) it would lead to a 7-hydroxycoumarin (IV). It has been found that chloroethylresorcin and chloropropylresorcin, the Clemmensen reduction products of chlororesacetophenone and chlororespropiophenone respectively, condense with acetoacetic ester or its alkyl derivatives forming compounds, which give intense yellow colour and do not show blue-violet fluorescence in alkaline solution characteristic of 7-hydroxycoumarins. Hence chlororesaceto- and propiophenones are respectively 5-chlororesacetophenone (I, R=Cl; R'=H) and 5-chlororespro-

piophenone (I, $R = Cl$; $R' = Me$). The preparation of the identical chlororesacetophenone by the chlorination of 4-ethylresorcin with sulphuryl chloride confirms the above constitution.



It should be observed that during the reduction of 5-chlororespropio-phenone by Clemmensen's method, along with chloropropylresorcin a halogen-free reduction product has been isolated which has been identified as 4-propylresorcin, since it produces a coumarin by condensation with acetoacetic ester, which is identical in all respects with 7-hydroxy-6-propyl-4-methylcoumarin, obtained from an authentic sample of 4-propylresorcin prepared by Clemmensen's reduction of respropio-phenone.

The removal of halogen atom in Clemmensen's reduction is found not to be unusual as in the case of bromoresacetophenone, the ketone derived from 4-bromoresorcin, a non-halogenated product has been obtained quantitatively. The latter has been identified to be 4-ethylresorcin, since it produces 7-hydroxy-4-methyl-6-ethylcoumarin, identical with the coumarin derived from an authentic sample of 4-ethylresorcin by condensation with acetoacetic ester. The isolation of 4-ethylresorcin from bromoresacetophenone proves definitely that the latter has the constitution (I, $R = Br$, $R' = H$).

It has been found during this investigation that the halogenated resaceto- and propiophenones do not condense with acetoacetic ester in the presence of condensing agents such as alcoholic sodium ethoxide, syrupy phosphoric acid, dry hydrochloric acid, piperidine and anhydrous aluminium chloride, the unchanged halogenated ketones being recovered in each case.

The study of the ketones from the halogenated resorcinols led us to prepare the ketones from 4-chlororesorcin (Chakravarti and Mukerjee, *loc. cit.*) and to elucidate their constitution in an analogous manner, but the results obtained are perplexing. 4-Chlororesorcin, when condensed with benzyl cyanide according to Hoesch's method, yields instead of a halogenated ketone a substance, which does not contain chlorine and which is not identical with the ketone obtained from resorcin and benzyl cyanide. The two substances differ widely and further the Clemmensen's reduction products are also different. The ketone from chlororesorcin and benzyl cyanide (needles) melts at 140° . It gives violet colour with ferric chloride and on Clemmensen's reduction gives a substance, m. p. 127° . The ketone from resorcin and benzyl cyanide (long flat prisms) melts at 160° . It does not give any colour with ferric chloride and the Clemmensen's reduction product melts at 72° , turning brown rapidly in air.

In the course of this investigation it was thought advisable to find out how the pyrone ring was affected under Clemmensen's condition. Coumarin itself, on Clemmensen's reduction, yields a crystalline substance (m. p. 235°). (Found: C, 72.83, 73.01; H, 4.67, 4.81; M.W. by Rast's method, 272). It has not been possible to assign any constitution to the reduction product but it may be a product of pinacolone type.

EXPERIMENTAL.

*5-Chlororesacetophenone.—4-Chlororesorcin (25 g.) was added to a solution of freshly fused zinc chloride (25 g.) in glacial acetic acid (25 c. c.) and the mixture heated to 145° for 3 minutes. The cold red syrupy mass was treated with concentrated hydrochloric acid (25 c. c.) and water (25 c. c.), when chlororesacetophenone separated as a yellow crystalline mass. It was collected and crystallised from hot water as needles, m. p. 171° , yield 10 g. It gives a violet colour with ferric chloride. It is readily soluble in alcohol, acetone, chloroform and acetic acid. (Found: Cl, 18.94. $C_8H_7O_3Cl$ requires Cl, 19.03 per cent).

The semicarbazone, prepared in the usual way, melts at 315° . (Found: N, 17.80. $C_8H_{10}O_3N_3Cl$ requires N, 17.25 per cent).

4-Chloro-6-ethylresorcin.—5-Chlororesacetophenone (5 g.), zinc amalgam (40 g.) and hydrochloric acid (1: 2, 120 c.c.) were refluxed for 4 hours with the addition of a fresh quantity of hydrochloric acid during the reduction. The greenish yellow solution was filtered and the filtrate after being saturated with common salt was extracted with ether, the ether evaporated, when an oil was obtained which on cooling and keeping in vacuum solidified to a mass of crystals. It crystallised from a small quantity of water as needles, m.p. 84° , yield 3 g. (Found: Cl, 20.69. $C_8H_9O_2Cl$ requires Cl, 20.5 per cent).

6 (or 8)-Chloro-5-hydroxy-4-methyl-8 (or 6)-ethylcoumarin.—4-Chloro-6-ethylresorcin (1 g.) and acetoacetic ester (1 g.) were condensed in the presence of sulphuric acid (2 c.c.) in the cold. The solution was kept overnight and poured into crushed ice and the crystalline product was recrystallised from rectified spirit as colourless prisms, m.p. 175° . It dissolves in caustic alkalis with intense yellow colour and does not show any fluorescence characteristic of 7-hydroxycoumarins. (Found: Cl, 14.61. $C_{12}H_{11}O_3Cl$ requires Cl, 14.8 per cent).

The *acetyl* derivative, prepared in the usual way by heating with acetic anhydride and sodium acetate, was crystallised from dilute alcohol in silky needles, m.p. 100° (Found: Cl, 12.55. $C_{14}H_{13}O_4Cl$ requires Cl, 12.65 per cent).

The identical coumarin (m.p. and mixed m.p. 175°) was obtained by heating 4-chloro-6-ethylresorcin (2 g.), acetoacetic ester (2 g.) and phosphorus pentoxide (10 g.) on a water-bath for $\frac{1}{2}$ hour (Simonis' reaction). The reaction mixture was treated with powdered ice and the oil separating was extracted with ether. On removing ether the oil was dissolved with 10% alkali and reprecipitated with hydrochloric acid. The process was repeated several times when the oil solidified on chilling. It crystallised from alcohol (charcoal).

6 (or 8)-Chloro-5-hydroxy-3 : 4-dimethyl-8 (or 6)-ethylcoumarin was obtained by condensing 4-chloro-6-ethylresorcin with α -methylacetoacetic ester in presence of sulphuric acid in the usual manner. It crystallised from alcohol in colourless needles, m.p. 183° . It dissolves in alkali with a yellow colouration. (Found: Cl, 13.91. $C_{13}H_{13}O_3Cl$ requires Cl, 14.05 per cent).

Formation of 4-Chloro-6-ethylresorcin by the Action of Sulphuryl Chloride on 4-Ethylresorcin.—Sulphuryl chloride (5 g.) was added drop by drop to ice-cooled ethereal solution of 4-ethylresorcin, obtained by the reduction of resacetophenone, when there was a copious evolution of hydrochloric acid and sulphur dioxide. The ethereal

solution was washed by sodium bicarbonate solution and then by water and the ether allowed to evaporate, when an oil was obtained which solidified on keeping and stirring. After removing the adhering tar on a porous plate it was crystallised from hot water (charcoal), m.p. 85° , yield 1 g. It is identical in all respects with 4-chloro-6-ethyl-resorcin, prepared above, and forms identical coumarin on condensation with acetoacetic ester.

5-Chlororespropio-phenone.—Chlororesorcin (25 g.) and a solution of freshly fused zinc chloride (25 g.) in propionic acid (25 g.) was boiled for 3 minutes. The red syrupy liquid on cooling was treated with powdered ice and hydrochloric acid, when an oil separated which solidified on chilling. It crystallised from dilute alcohol (charcoal) as yellow needles, m.p. 90° , yield 10 g. It gives a violet colour with ferric chloride and is soluble in boiling water. (Found: Cl, 17.29. $C_9H_7O_3Cl$ requires Cl, 17.70 per cent).

Clemmensen's Reduction of 5-Chlororespropio-phenone : Formation of 4-Chloro-6-propylresorcin and 4-Propylresorcin.—5-Chlororespropio-phenone (5 g.), zinc amalgam (20 g.), hydrochloric acid (30 c.c.) and water (60 c.c.) were refluxed for 5 hours. The yellow solution was filtered hot, when crystals separated on cooling. It was crystallised from luke warm water as colourless needles, m.p. 63° , yield 3 g. It gives a blue colour with ferric chloride. (Found: Cl, 18.79. $C_9H_{11}O_2Cl$ requires Cl, 19.03 per cent).

The mother-liquor after the separation of 4-chloro-6-propylresorcin was saturated with common salt and extracted with ether, the ethereal extract dehydrated by calcium chloride and ether evaporated. The oil separating solidified on scratching. It does not contain chlorine and is found to be identical with propylresorcin, prepared by the reduction of respropio-phenone (*vide infra*).

Clemmensen's Reduction of Respropio-phenone.—Respropio-phenone (5 g.), concentrated hydrochloric acid (20 c.c.), water (40 c.c.), zinc amalgam (20 g.), alcohol (10 c.c.) were refluxed for 5 hours. The filtered solution was saturated with common salt, extracted with ether, ethereal extract was dehydrated and ether evaporated, when an oil was obtained which solidified on chilling. It crystallised in colourless needles from petroleum ether (b.p. 50°), m.p. 77° , yield 2 g. (Found: C, 70.78; H, 7.8. $C_9H_{12}O_2$ requires C, 71.05; H, 7.89 per cent).

Condensation of Propylresorcin with Ethyl Acetoacetate : Formation of 6-Propyl-4-methyl-7-hydroxycoumarin.—6-Propylresorcin, obtained by the reduction of respropio-phenone or 5-chlororespropio-phenone, was

condensed with ethyl acetoacetate using sulphuric acid in the usual manner. The product crystallised from alcohol as needles, m.p. 174° . (Found : C, 71.48; H, 6.29. $C_{13}H_{14}O_3$ requires C, 71.55; H, 6.42 per cent).

6-(or 8)-Chloro-5-hydroxy-4-methyl-8 (or 6)-propylcoumarin, the condensation product of 5-chlororespropiphenone and ethyl acetoacetate in the presence of sulphuric acid, crystallised from alcohol as prisms, m.p. 185° . It is soluble in alkalis with intense yellow colour and does not show any blue-violet fluorescence characteristic of 7-hydroxycoumarins. (Found : Cl, 14.22. $C_{13}H_{13}O_3Cl$ requires Cl, 14.05 per cent).

* 5-Bromoresacetophenone.—Bromoresorcin (5 g.) and a solution of fused zinc chloride (5 g) in glacial acetic acid (5 g) were boiled for 3 minutes. The cold syrupy mass was treated with water acidulated with hydrochloric acid, when bromoresacetophenone separated. It was collected and crystallised from hot water, m.p. 167° . (Found : Br, 34.36. $C_8H_7O_3Br$ requires Br, 34.63 per cent).

The semicarbazone crystallised from alcohol, m.p. 255° . (Found N, 14.96. $C_8H_{10}O_3N_3Br$ requires N, 14.59 per cent).

Clemmensen's Reduction of 5-Bromoresacetophenone: Formation of 4-Ethylresorcin.—5-Bromoresacetophenone (2 g.), zinc amalgam (20 g.), concentrated hydrochloric acid (20 c.c.) and water (40 c.c) were refluxed for 4 hours. The solution was filtered and concentrated to half its volume and saturated with common salt and extracted with ether. On evaporation of ether an oil was obtained, which solidified on scratching. It crystallised from chloroform, m.p. 97° . It has been identified as 4-ethylresorcin as it does not depress the m.p. of 4-ethylresorcin obtained by reduction of resacetophenone and also forms the identical coumarin by its condensation with ethyl acetoacetate in the presence of sulphuric acid.

7-Hydroxy-6-ethyl-4-methylcoumarin.—The condensation product of 4-ethylresorcin and ethyl acetoacetate using sulphuric acid in the usual manner crystallised from alcohol (charcoal) as needles, m.p. 210° . (Found : C, 70.86; H, 5.71. $C_{12}H_{12}O_3$ requires C, 70.60; H, 5.8 per cent).

The Ketone obtained by Hoesch's Reaction of 4-Chloroorcin with Benzyl Cyanide and its Clemmensen's Reduction Product.—Dry hydrochloric acid gas was passed into a dry ethereal solution of chloroorcin (10 g.) and benzyl cyanide (7.5 g.) containing fused zinc chloride (4 g.) and the solution cooled in ice. The mixture was allowed to stand for 2 days and the syrupy mass extracted with ether and the aqueous layer heated on a water-bath, when a brown oil separated, which was dissolved in ether. The ether was distilled off, when the oil solidified on standing for several days.

It was washed by sodium bicarbonate solution and then crystallised from dilute alcohol in needles, m.p. 140° , yield 3 g. It does not contain chlorine. It is soluble in boiling water and is moderately soluble in alcohol. It is soluble in alkalis and gives a violet colour with ferric chloride. (Found : C, 74.18; H, 5.7. $C_{15}H_{14}O_3$ requires C, 74.3; H, 5.7 per cent).

The above ketone (3 g.), hydrochloric acid (1:2, 60 c.c.), and zinc amalgam (20 g.) were refluxed for 8 hours. The melted product separating was separated and crystallised from hot water in needles, m.p. 127° , yield 1 g. It gives no colour with ferric chloride. It is soluble in alkalis forming a colourless solution. (Found : C, 77.1; H, 6.3 per cent).

The Ketone obtained by Hoesch's Reaction of Orcin with Benzyl Cyanide and its Clemmensen's Reduction Product—Dry hydrochloric acid gas was passed into a cold ethereal solution of orcin (10 g) and benzyl cyanide (10 g) containing fused zinc chloride (4 g.) and the mixture was set aside for 2 days. The syrupy mass was shaken with ether, when the imino-hydrochloride separated. It was collected and heated with water on the water-bath, when the ketone separated as flat prisms, m.p. 160° , yield 5 g. It crystallises from dilute alcohol. It is soluble in alkalis and does not give any colour with ferric chloride. (Found: C, 72.98; H, 5.78 per cent).

The ketone (2 g.), zinc amalgam (20 g.) and hydrochloric acid (1:2, 60 c.c.) were refluxed for 8 hours. The solution was filtered hot and on cooling crystals separated. These were collected and crystallised from hot water, m.p. 72° , yield 1.5 g. It turns brown on exposure to air. It does not give any colour with ferric chloride and does not undergo Pechmann's reaction. (Found: C, 79.58; H, 7.44 per cent).

Reduction of Coumarin by Clemmensen's Method.—Coumarin (5 g.), zinc amalgam (20 g.), hydrochloric acid (1:2, 60 c.c.) were refluxed for 4 hours with the addition of 60 c.c. more of hydrochloric acid (1:2) gradually during the reduction. The cold solution was filtered and the solid on the filter paper was crystallised from glacial acetic acid as colourless needles, m.p. 235° , yield 2 g. of the pure product. It is insoluble in alkali and does not decolourise bromine. [Found : C, 72.83, 73.01; H, 4.67, 4.81; M.W., (Rast's method), 272].

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Received January 11, 1939.

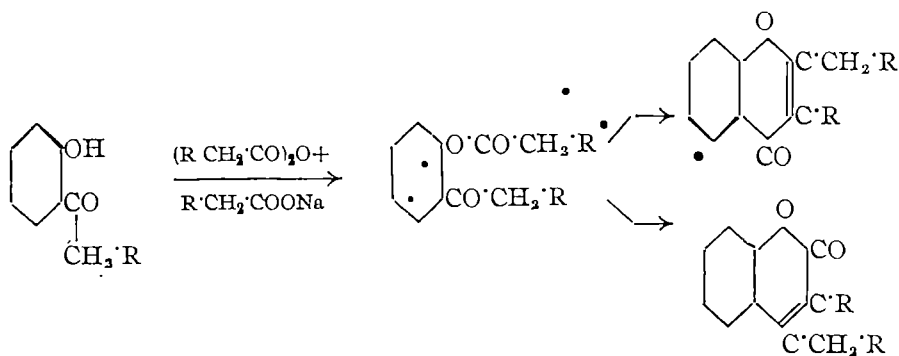
* The compounds marked with an asterisk (*) have been prepared in collaboration with Sailendra Mohon Mukerjee.

LIMITED APPLICABILITY OF KOSTANECKI'S REACTION. THE INFLUENCE OF HALOGEN ATOM ON THE REACTION.

BY DUHKHAHARAN CHAKRAVARTI AND BROJESWAR MAJUMDAR.

The ketones, *e g*, 5-chloro-3-methyl-2-hydroxyacetophenone, 3-chloro-5-methyl-2-hydroxyacetophenone, 5-chloro-3-methyl-2-hydroxypropio-phenone, 3-chloro-5-methyl-2-hydroxypropio-phenone, 5-chloro-2-hydroxypropio-phenone and 5-bromo-2-hydroxypropio-phenone have been submitted to Kostanecki's reaction with (a) acetic anhydride and sodium acetate, (b) propionic anhydride and sodium propionate and (c) butyric anhydride and sodium butyrate and it has been found that the *o*-hydroxyacetophenones yield with sodium propionate and propionic anhydride coumarins and in other cases chromones are obtained.

The formation of an α -pyrone derivative in Kostanecki's reaction is not unusual as the intermediate acyl derivative may lose water yielding either an α -pyrone or a γ -pyrone (Wittig, Baugart and Richter, *Annalen*, 1925, **446**, 155; Bargellini, *Atti. R. Accad. Lincei*, 1925, **2**, 261; Baker and Eastwood, *J. Chem. Soc.*, 1929, 2906; Chadha, Mahal and Venkataraman, *ibid.*, 1933, 1460; Heilbron *et. al.*, *J. Chem. Soc.*, 1933, 1263; 1934, 1311, 1581; 1936, 295; *cf.* Chakravarti and Bagchi, *J. Indian Chem. Soc.*, 1936, **13**, 689; Flynn and Robertson, *J. Chem. Soc.*, 1936, 215).



In studying Simonis' reaction Chakravarti and co-workers found that the halogen atoms favoured the formation of chromones in monohydric

phenols and the present investigation was undertaken with a view to study Kostanecki's reaction on the halogenated aceto-, propio-, and butyrophenones and to find out whether the halogen atoms have any marked influence on the formation of γ -pyrones as in Simonis' reaction. The ketones, *e.g.*, 5-chloro-3-methyl-2-hydroxyacetophenone, 3-chloro-5-methyl-2-hydroxyacetophenone, 5-chloro-3-methyl-2-hydroxypropio-phenone, 3-chloro-5-methyl-2-hydroxypropio-phenone, 5-chloro-2-hydroxypropio-phenone and 5-bromo-2-hydroxypropio-phenone have been submitted to Kostanecki's reaction with (a) acetic anhydride and sodium acetate, (b) propionic anhydride and sodium propionate, and (c) butyric anhydride and sodium butyrate.

The ketones have been prepared from the acetyl and propionyl derivatives of the halogenated phenols by heating with aluminium chloride (Fries' rearrangement). In course of the preparation of the above ketones it has been observed that in some cases an appreciable amount of the phenol was formed due to the deacylation but the formation of phenol was effectively overcome by heating the mixture of acetyl or propionyl derivative and aluminium chloride for $\frac{1}{2}$ –1 hour at 120° and not for only 10 minutes as recommended by Rosenmund and Schnurr (*Annalen*, 1928, 460, 84). In attempting to prepare the *o*-hydroxybutyrophenones from the butyryl derivatives of the phenols, it has been found that variation of temperature and the increase of the duration of baking with aluminium chloride does not increase the yield of the butyrophenone, which is always obtained in very poor yield and is contaminated with the phenol. In the case of *o*-chlorophenol propionate and *o*-bromophenol propionate, the propionyl group migrates to the *para* position to the hydroxyl group since the acetyl derivative is formed on acetylation with acetic anhydride and not *o*- or γ -pyrone as is the case with the *o*-hydroxyketones.

On heating any of the above *o*-hydroxyacetophenones with sodium acetate and acetic anhydride 3-acetylchromones are obtained. In order to establish the γ -pyrone structure of the products attempts have been made to condense the resulting 2-methylchromones with benzaldehyde in presence of sodium ethoxide (*cf.* Heilbron, Barnes and Morton, *J. Chem. Soc.*, 1923, 123, 2569; Chakravarti, *J. Indian Chem. Soc.*, 1931, 8, 129) in view of the reactivity of the 2-methyl group in γ -pyrone, but the styryl derivative is not formed. The 3-acyl group probably produces hindrance in the reactivity of the 2-methyl group. That the products obtained are not coumarins but chromones is proved by Wittig's method (Wittig, *Ber.*, 1924, 57, 88; Heilbron, Hey and Lythgoe, *J. Chem. Soc.*, 1934, 1581). The absence of

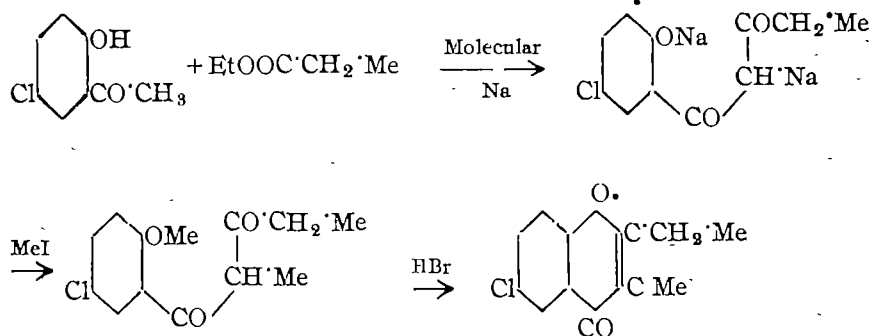
any non-acylated chromone in the product is shown by the fact that no oxonium salt is obtained by passing hydrochloric acid gas into an ethereal solution of the substance (cf. Wittig, *Annalen*, 1925, **446**, 155).

The *o*-hydroxyacetophenones yield with sodium propionate and propionic anhydride coumarins, which are identical with the coumarins described by Chakravarti and Banerjee (*J. Indian Chem. Soc.*, 1936, **13**, 619) from the appropriate phenols and ethyl α -methylacetoacetate by Pechmann's reaction.

The *o*-hydroxypropiophenones, when heated with acetic anhydride and sodium acetate, give exclusively chromones and not coumarins and the resulting 2-methylchromones are readily characterised by the formation of 2-styryl derivatives on condensation with benzaldehyde in the presence of sodium ethoxide. Similarly the products obtained by heating *o*-hydroxypropiophenones with sodium propionate and propionic anhydride are also chromones and no trace of coumarin could be detected by Wittig's method of separation of coumarins from chromones. It should, however, be noted that the 2-ethylchromones thus obtained, resist the formation of styryl derivatives with benzaldehyde or piperonal even under the experimental conditions recommended by Heilbron, Hey and Lowe (*J. Chem. Soc.*, 1934, 1311). Interesting results have been obtained while applying the standard method of separation of coumarins from chromones. 6-Chloro-2-ethyl-3 : 8-dimethylchromone, under Wittig's condition, regenerates the parent ketone, 5-chloro-3-methyl-2-hydroxypropiophenone, instead of the expected diketone. The isolation of the parent *o*-hydroxyketone establishes the γ -pyrone structure of the condensation product, as the alkaline hydrolytic fission of the chromones only and not of the coumarins can explain the formation of the original ketone.

In view of the non-reactivity of the 2-ethylchromones, described in this paper, towards benzaldehyde, 2-ethylchromones and the isomeric 4-ethylcoumarins, which are the possible products of the reaction, have been synthesised by unambiguous methods. Ethyl propionate has been condensed with the *o*-hydroxyacetophenone in the presence of molecular sodium (cf. Kostanecki *et al.*, *Ber.*, 1900, **33**, 330, 471; 1901, **34**, 2942 *et. seq.*; Wittig *et al.*, *Ber.*, 1924, **57**, 88; *Annalen*, 1925, **446**, 155; Baker, *J. Chem. Soc.*, 1933, 1388) and the sodium salt of the diketone, thus obtained, is methylated directly and the final ring-closure, which involves the elimination of methyl alcohol, is effected by hydrobromic acid in glacial acetic acid solution. Thus the product, obtained from 5-chloro-2-hydroxyacetophenone and ethyl propionate, is identical in all respects with the

compound prepared from 5-chloro-2-hydroxypropiophenone and propionic anhydride and sodium propionate.



The *o*-hydroxypropiophenone does not undergo condensation with ethyl propionate and hence the condensation product of *o*-hydroxyacetophenone with ethyl propionate has to be methylated.

The condensation products of the halogenated *o*-hydroxypropiophenones with sodium butyrate and butyric anhydride are also γ -pyrones and like the 2-ethylchromones the 2-propylchromones also do not give benzyldene derivative and regenerate the parent ketone by alkaline hydrolytic fission under Wittig's experimental condition.

The isomeric 4-ethylcoumarins, which are the alternative possible products of the above reaction, have also been synthesised (Chakravarti and Majumdar, *J. Indian Chem. Soc.*, 1938, **15**, 136).

Thus a study of Kostanecki's reaction on the halogenated *o*-hydroxyaceto- and propiophenones has revealed no marked influence of the halogen atom on the formation of γ -pyrones as in Simonis' reaction.

EXPERIMENTAL.

5-Chloro-3-methyl-2-hydroxyacetophenone.—The acetate of 4-chloro-2-methylphenol (20 g., b.p. 235°), prepared by the action of acetyl chloride on the phenol in the usual manner, was mixed with finely powdered aluminium chloride (20 g.) and the mixture heated at 120° for 20-30 minutes. The glassy mass was decomposed with dilute hydrochloric acid and distilled in steam. The product obtained from the steam distillate was crystallised from dilute alcohol as light yellow needles, m.p. 70°, yield 15 g. (Found : Cl, 19.24. C₉H₉O₂Cl requires Cl, 19.38 per cent).

The *semicarbazone*, prepared in the usual way, crystallised from alcohol, m.p. 283° (decomp.). (Found: N, 17.9. $C_{10}H_{12}O_2N_3Cl$ requires N, 17.4 per cent).

5-Chloro-3-methyl-2-hydroxypropio-phenone.—The propionate of 4-chloro-2-methylphenol (20 g., b.p. $249-252^{\circ}$), prepared by the action of propionyl chloride on the phenol, was heated for $\frac{1}{2}$ hour at 120° with aluminium chloride (20 g.). The mixture was treated with dilute hydrochloric acid and distilled in steam, and the solid obtained from the distillate was crystallised from dilute alcohol as colourless needles, m.p. 61° , yield 17 g. (Found: Cl, 17.90. $C_{10}H_{11}O_2Cl$ requires Cl, 17.88 per cent).

The *semicarbazone* crystallised from alcohol, m.p. 205° . (Found: N, 16.30. $C_{11}H_{14}O_2N_3Cl$ requires N, 16.44 per cent).

3-Chloro-4-hydroxypropio-phenone was prepared from the propionate of *o*-chlorophenol (20 g., b. p. $230-34^{\circ}$) by heating with aluminium chloride (20 g.) for $\frac{1}{2}$ hour. It was purified by distillation in steam and crystallised from benzene as colourless plates, m. p. 80° . (Found: Cl, 18.73. $C_9H_9O_2Cl$ requires Cl, 19.24 per cent).

The *acetyl* derivative, prepared with sodium acetate and acetic anhydride, was obtained as a colourless oil, b.p. $155^{\circ}/6$ mm., which solidifies on keeping to colourless needles. (Found: Cl, 15.63. $C_{11}H_{11}O_3Cl$ requires Cl, 15.67 per cent).

3-Bromo-4-hydroxypropio-phenone was prepared by heating the propionate of *o*-bromophenol (20 g., b. p. $245-60^{\circ}$) with aluminium chloride (20 g.) for $\frac{1}{2}$ hour. It was finally purified by distillation in steam. It crystallised from benzene, m.p. 130° . (Found: Br, 34.65. $C_9H_9O_2Br$ requires Br, 34.93 per cent).

5-Bromo-2-hydroxypropio-phenone, obtained in the usual manner from the propionate of *p*-bromophenol (20 g., b.p. 250°) by heating with aluminium chloride (30 g.) for 30-40 minutes, was distilled in steam and crystallised from benzene, m.p. 78° , yield 16 g. (Found: Br, 34.72. $C_9H_9O_2Br$ requires Br, 34.93 per cent).

*General Method for the Acetylation, Propionylation and Butyrylation
of the Aceto- and Propio-phenones.*

A mixture of the *o*-hydroxyketone (4 g.), freshly fused sodium salt of the fatty acid (6-10 g.) and the anhydride of the acid (30-40 g.) was heated at $170-180^{\circ}$ for about 12 hours. The reaction mixture was then

poured into water and heated on the water-bath for $\frac{1}{2}$ hour to decompose the excess of acid anhydride and the solid was collected and crystallised from a suitable solvent. The products obtained by the acetylation of *o*-hydroxyacetophenones and *o*-hydroxypropiophenones are described in Table I and the compounds prepared by the propionylation of *o*-hydroxy-aceto- and propiophenones are described in Table II. Table III describes the compounds obtained by the butyrylation of *o*-hydroxypropiophenones.

Alkaline Hydrolysis of 6-Chloro-3 : 8-dimethyl-2-ethylchromone : Isolation of 5-Chloro-2-hydroxy-3-methylpropiophenone.—6-Chloro-2-ethyl-3 : 8-diethylchromone was dissolved in alcoholic sodium ethoxide solution and left overnight. The insoluble residue melted at 85° (mixed m.p. with the chromone). The solution was acidified with hydrochloric acid and extracted with ether and the ether extract was repeatedly washed with cold alkali (5%) and then with water. On acidifying the alkaline wash with hydrochloric acid a solid was obtained which did not respond to the test of 1 : 3-diketone nor did it regenerate the original chromone on heating with glacial acetic acid and hydrochloric acid. It gives a deep violet colouration with ferric chloride and on crystallisation was found to be identical with 5-chloro-2-hydroxy-3-methylpropiophenone (m.p. and mixed m.p. 61°).

Synthesis of 6-Chloro-3-methyl-2-ethylchromone from 5-Chloro-2-hydroxyacetophenone and Ethyl Propionate.—A mixture of 5-chloro-2-hydroxyacetophenone (9.5 g.) and ethyl propionate (22 g.) was added to molecular sodium (3.5 g.), the reaction flask being cooled in ice. The mixture was then heated for 1 hour on the water-bath, cooled and poured into powdered ice. The sodium salt separating was collected, washed with ether and dried. It was then suspended in dry acetone and methylated with the requisite quantity of methyl iodide. The excess of acetone was then distilled off and the residual oil was poured into water, extracted with ether, the ethereal extract was dried and ether removed, when a sticky mass was obtained. It was dried in a vacuum desiccator and then heated with acetic acid and hydrobromic acid (1 c.c.) for 1 hour on the water-bath. The mixture was then poured into water, when an oil separated which gradually solidified to a brown mass. It was crystallised from dilute alcohol and found to be identical with the product obtained by the interaction of 5-chloro-2-hydroxypropiophenone, propionic anhydride and sodium propionate (m.p. and mixed m.p. 123°).

TABLE I.
Acetylation of o-Hydroxyaceto- and propiophenones.

Name of the compound.	Molecular formula.	Ketone used.	M.p	Analysis Found ;	Calc.	Remarks
6-Chloro-2 : 8-dimethyl-3-acetylchromone	$C_{15}H_{11}O_3Cl$	5-Chloro-3-methyl-4-hydroxyacetophenone	139°	Cl, 14.4%	Cl, 14.177%	Colourless plates from alcohol.
8-Chloro-2 : 6-dimethyl-3-acetylchromone	$C_{15}H_{11}O_3Cl$	5-Methyl-3-chloro-2-hydroxy acetophenone	131°	Cl, 13.75	14.17	Colourless plates from alcohol
6-Chloro-2 : 3 : 8-trimethylchromone	...	5-Chloro-3-methyl-2-hydroxypropiophenone	124°			Needles from alcohol (identical with the compound described by Chakravarti and Banerjee, <i>loc. cit.</i>)*
—Styryl derivative**	166-167°			
8-Chloro-2 : 3 : 6-trimethylchromone	...	5-Methyl-3-chloro-2-hydroxypropiophenone	154-156°			
—Styryl derivative**	183°			
6-Chloro-2 : 3-dimethylchromone	...	5-Chloro-2-hydroxypropiophenone	86-87°			Identical with the compound described by Wittig (<i>Annalen</i> , 1925, 246 , 186).
—Styryl derivative	144-145°			
6-Bromo-2 : 3-dimethylchromone	...	5-Bromo-2-hydroxypropiophenone	113°			Identical with the compound prepared by Simonis from <i>p</i> -bromophenol and ethyl α -methyl acetate

* The m.p. of 6-chloro-2 : 3 : 8-trimethylchromone was recorded as 113° by Chakravarti and Banerjee, but the compound prepared again by the application of Simonis' reaction on 4-chloro-2-methylphenol and ethyl α -methyl-acetoacetate was found to melt at 124°.

** *cf.* Chakravarti and Banerjee, *loc. cit.*

TABLE II.
Propionylation of o-Hydroxyaceto- and propiophenones.

Name of the compound.	Molecular formula.	Ketone used	M. p.	Found	Analysis	Remarks.
6-Chloro-3:4:8-trimethyl- coumarin	$C_{13}H_{11}O_2Cl$	5-Chloro-3-methyl- 2-hydroxyaceto- phenone	94°	Cl, 15.64%	Cl, 15.95%	Pale yellow needles from alcohol (b.p. 180-200°/6mm).
8-Chloro-3:4:6-trimethyl coumarin	$C_{13}H_{11}O_2Cl$	5-Methyl-3-chloro-2- hydroxyaceto- phenone	157°	15.64	15.95	Identical with the com- pound prepared by Chakravarti and Baner- jee (<i>loc. cit.</i>) from 2- chloro-4-methylphenol and ethyl α -methyl- acetoacetate
6-Chloro-3:8-dimethyl- 2-ethylchromone	$C_{13}H_{13}O_2Cl$	5-Chloro-3-methyl-2- hydroxypropio- phenone	85°	15.3	15.0	Does not give styryl derivative. It regener- ates 5-chloro-3-methyl-2- hydroxypropiophenone on hydrolysis.
8-Chloro-3:6-dimethyl- 2-ethylchromone	$C_{13}H_{13}O_2Cl$	5-Methyl-3-chloro-2- hydroxypropio- phenone	74-75°	15.2	15.0	Needles from alcohol It gives 5-methyl-3- chloro-2-hydroxypro- piophenone on hy- drolysis
6-Chloro-2-ethyl-3-methyl- chromone	$C_{13}H_{11}O_2Cl$	5-Chloro-2-hydroxy- propiophenone	65-66°	15.7	15.9	Needles from alcohol. It is identical with the chromone obtained from 5-chloro-2-hydroxy- acetophenone and ethyl propionate <i>vide supra</i> .
6-Bromo-2-ethyl-3-methyl- chromone	$C_{13}H_{11}O_2Br$	5-Bromo-2-hydroxy- propiophenone	87°	Br, 29.87	Br, 29.96	Colourless needles from alcohol.

LIMITED APPLICABILITY OF KOSTANECKI'S REA

TABLE III.
Butyrylation of o-Hydroxypropio-phenones.

Name of the compound.	Molecular formula	Ketone used	M. p	Found.	Analysis.	Remarks.
6-Chloro-3:8-dimethyl-2-propylchromone	$C_{14}H_{16}O_2Cl$	5-Chloro-3-methyl-2-hydroxypropio-phenone	95°	Cl, 13.85%	Cl, 14.17%	Crystallised from alcohol.
8-Chloro-2-propyl-3:6-dimethylchromone	$C_{14}H_{16}O_2Cl$	5-Methyl-3-chloro-2-hydroxypropio-phenone	68-71°	14.18	14.17	Colourless needles from alcohol. It regenerates 5-methyl-3-chloro-2-hydroxypropio-phenone on hydrolysis.
6-Chloro-2-propyl-3-methylchromone	$C_{13}H_{13}O_2Cl$	5-Chloro-2-hydroxypropio-phenone	85°	15.09	15.00	Colourless plates from alcohol
6-Bromo-2-propyl-3-methylchromone	$C_{13}H_{13}O_2Br$	5-Bromo-2-hydroxypropio-phenone	83-84°	Br, 28.0	Br, 28.48	Needles from alcohol

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Received January 11, 1939

A NOTE ON METHYL β -RESORCYLATE.

By S. RANGASWAMI.

Robinson and Shah (*J. Chem. Soc.*, 1934, 1496) observed that methyl β -resorcyate crystallised from aqueous methyl alcohol or chloroform with some solvent of crystallisation which was gradually lost on exposure to air or more rapidly on heating and that after drying in *vacuo* at 60-70° it melted at 118-19°. It is now found that the ester obtained from β -resorcylic acid and methyl alcoholic hydrogen chloride or methyl alcoholic sulphuric acid melts at 78-80° when freshly prepared. Crystallisation from aqueous methyl or ethyl alcohol or air-drying during a period of 3 weeks does not affect the m.p. On the other hand crystallisation from chloroform gives a product melting indefinitely between 85° and 110°. Further, when the air-dried material is left overnight in a sulphuric acid desiccator or dried at 80° for 1 hour, it loses about 10% of its weight and is converted into a brittle powder, m.p. 119-20°. This higher melting compound analyses correctly for $C_8H_8O_4$ (methyl resorcyate). Crystallisation from anhydrous solvents like benzene and chloroform leaves it unchanged, but crystallisation from aqueous methyl alcohol or dilute acetic acid lowers the m.p. The specimen obtained from the former solvent melts at 78-80° and analysis indicates that it is the monohydrate of methyl β -resorcyate. (Found : C, 51.2 ; H 5.2, loss on drying at 80°, 10.0%. Calc. for $C_8H_8O_4 \cdot H_2O$: C, 51.6; H, 5.4; loss on drying, 9.7 per cent).

CHEMICAL LABORATORIES,
ANDHRA UNIVERSITY,
WALTAIR

Received January 9, 1939.

A NOTE ON THE ANALYSIS OF CERTAIN ALGÆ.

BY M. NARASIMHAM AND S. N. PAL.

Though some work on drift sea-weed collected from the various places along the coast line of the Bombay Presidency was done (Dixit, *J. Indian Chem. Soc.*, 1930, **12**, 959), no work seems to have been made hitherto on the drift sea-weeds collected along the Coromandal coast. The authors collected four species of sea-weeds from different places in the Madras Presidency during the winter months of 1930. The result of analysis of air-dried samples is tabulated below.

Weed.	Cellulose.	Protein.	Iodine.	Place.
Padina	18.7 %	15.2 %	Nil	Bhimilipatam (Vizagapatam District).
Ulva	31.0	1.0	Nil	Vizagapatam.
Enteromorpha	24.4	2.6	Nil	Vizagapatam.
Red algae (Poly-siphonia)	18	7.75	0.044 %	Pamban (Ramanad District).

Our thanks are due to Mr. V. Venkateswarlu, M.Sc. of the Botany Department, P. R. College for taking the trouble of identifying each species.

CHEMICAL LABORATORY,
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COCANADA.

Received February 25, 1939

REVIEWS

An Introduction to the Chemistry of Cellulose—By J. T. MARSH & F. G. WOOD (WITH A FOREWORD BY SIR KENNETH LEE). PUBLISHED BY CHAPMAN & HALL LTD., LONDON, 1938. DERM 1620. PP. 431 + XV, PRICE 21SH. NET.

The present volume fills up an important gap created by constant research and will be welcomed by all who find it difficult to keep pace with the progress of science in the field of cellulose chemistry. The publication is a well-printed volume of 431 pages and is divided into five parts dealing with properties of cellulose, dispersed cellulose, modified cellulose, derivatives of cellulose and constitution and structure of cellulose.

The authors have performed in an admirable way the difficult task of arranging the vast mass of useful information in a systematic form, correlating them and presenting them in a concise and readable manner. Numerous references to recent publications and patent literature and some practical details of laboratory and large-scale methods have enhanced the usefulness of the book.

Defects are very few. In a few instances references to original literature are incomplete. It is regrettable that the work of Indian investigators has not received due recognition. Though the later work of Sakurada on ethers of hydroxy-acids has received attention, the earlier work of Chowdhury on the subject (*Biochem.*, Z, 1924, 148, 76-97) has escaped notice. Similarly the publication in this Journal (1936, 13, 294) on the molecular size of cellulose by surface tension method has not been noticed and some important contributions on the properties of cotton fibre made from Bombay Cotton Research Institute have also escaped attention. Views on molecular weight and fine structure have been incorporated but the relationship of the properties of cellulose fibres with molecular size and structure and the influence of accompanying impurities on fibre properties have not received due importance.

Apart from these minor defects, the volume presents a very valuable collection of useful information before the public and will be welcomed by all interested either in research on cellulose or in the processing of textile fibres. It is pleasant to find this volume gracefully dedicated to 'those who have done the work described in these pages.'

J. K. C.

The German Primer for Science Students—By HARAGOPAL BISWAS.
PUBLISHED BY THE UNIVERSITY OF CALCUTTA. PP. 258 + xvii, ROYAL
8vo.

The desirability, even necessity, of having a fair working knowledge of the German language on the part of students going in for higher studies in science has been recognised in every country, including ours. But the problem of acquiring that knowledge bristles with difficulties, in as much as German grammar is not easy to master, and then again, as scientific and idiomatic language differ greatly it is not nearly enough for a student of science to learn the language from the humanistic standpoint. The needs of a science student in this respect are therefore somewhat different from those of a student of any subject coming under the category of the humanistic group of studies.

The author has attempted to solve the problem by providing a special primer for science students, which though imperfect in many details, should nevertheless find a ready welcome by those for whom it is meant. What gives the book an added interest and value is that it contains the results of the author's own personal experience of learning German, of the difficulties he encountered in the process, and how he succeeded in overcoming them. The numerous passages taken out of scientific journals will serve to familiarise the student with the scientific language and terminology and help him to "get at it."

A. N. B.

FORMATION OF PERIODIC PRECIPITATE IN THE ABSENCE OF A FOREIGN GEL. PART II. FERRIC HYDROXIDE SOL BY DIFFERENT METHODS.

By R. N. MITTRA.

Ferric hydroxide sols have been prepared by acetate, carbonate and Krecke's methods for the study of periodic precipitation by the process of coagulation of these sols. The adsorption of sol by its own precipitate, the nature of the coagula which settle periodically and the speed of coagulation of the sols have been investigated. It has been shown that in obtaining the rings of precipitate by the coagulation of a sol, the adsorption of the sol by its own precipitate is not the only factor controlling the process.

In a preliminary study (Mittra, *Proc. Nat. Acad. Sci.*, 1936, **6**, 322) it was emphasised that for the production of periodic precipitate in sol by the process of coagulation, the adsorption of sol by its own precipitate, the nature of coagulum which settles periodically and also the speed of coagulation of the sol are the three important factors which determine the phenomenon. It is well known that the nature of the coagula, obtained by the addition of electrolytes to the sols of ferric hydroxide prepared by different methods, remarkably varies. Hence in order to study how far (i) the nature of coagulum, (ii) the speed of coagulation, and (iii) the adsorption of colloid by its own precipitate, control the process of the formation of rings, it has been necessary to have a systematic study of these and the results obtained on such investigations are detailed below.

With the object of comparing the sols of ferric hydroxide prepared by acetate, carbonate and Krecke's methods for the production of periodic precipitates, the sols of different degrees of purity were prepared. The original sols were diluted to the desired extents and these diluted sols were coagulated by mono- and bivalent electrolytes and the conditions of the coagula were noted after 24 hours. The volume of coagula, which determines the formation of rings, was found out by centrifuging for 5 minutes, 9 c.c. of the suspension obtained on thorough shaking of the coagula which settled periodically, out of the total volume of 20 c.c. of the sol-electrolyte mixture. It was observed that some of the precipitate which settled completely was peptised during the process of centrifuging. The amount of the precipitate thus peptised was estimated in each case. For finding the speed of coagulation, the sols were maintained at the concentration of 0.07378 g.

atom of Fe per litre. 10 C.c. of this sol were mixed with electrolytes, the total volume was made upto 20 c.c. and the time for complete coagulation noted. 8 C.c. of the sol-electrolyte mixture out of the total volume of 20 c.c. from such another set containing 0.000295 g. atom of Fe were taken in separate similar test tubes and set aside. The amounts of the uncoagulated sol from each of these 8 c.c. tubes were estimated successively at definite intervals of time by centrifuging the tubes for 5 minutes. The speed of the centrifuge was maintained at 2000 revolutions per minute. The coagulation-velocity curves have been drawn (not shown).

EXPERIMENTAL.

Ferric Hydroxide Sol (Acetate Method).

The sol was prepared (cf. Mittra and Dhar, *J. Indian Chem. Soc.*, 1932, 9, 315) and dialysed hot for 6 hours. It contained 0.25 g. atom of Fe per litre and its purity was 0.57.

TABLE I.

Sol conc. = 0.05 g. of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c. of which 9 c.c. contain 0.000225 g. atom of Fe.

Amount of electrolyte.	Volume of coagulum.	Amount of precipitate peptised.
<i>2N-KCl.</i>		
6.5 c.c.	0.30 c.c.	0.0000112 g. atom.
6.0	0.35	0.0000134
5.5	0.40	0.0000223
5.0	0.40	0.0000223
<i>N/40-K₂SO₄.</i>		
2.5	0.25	0.0000223

With this concentration of the sol, only broken bands appeared with KCl; with K₂SO₄ as coagulant, an indistinct evidence for the bands was there. With higher concentration of the sol, the coagula were in gel state and no settling occurred after 24 hours.

TABLE II.

Speed of coagulation.

5 C.c. of 2N-KCl (made upto 10 c.c.) coagulated 10 c.c. of the sol in 1 hour. (Ppt conc.).		7 C.c. of 2N-KCl (made upto 10 c.c.) coagulated 10 c.c. of the sol in 30 minutes.		4 C.c. of N/40-K ₂ SO ₄ (made upto 10 c.c.) coagulated 10 c.c. of the sol almost instantaneously.	
Time.	Amount left in sol state out of 0.000295 g atom of Fe.	Time.	Amount left in sol state out of 0.000295 g. atom of Fe.	Time.	Amount left in sol state out of 0.000295 g atom of Fe.
After 2 min.	No coagulation	After 2 min.	0.000256	After 2 min.	0.00002
15	0.000284 g. atom	15	0.000176	15	0.000014
45	0.00010	40	0.000048	40	0.000014
60	0.000048	60	0.000048		

The original sol was further purified by hot dialysis for 3 hours and contained 0.268 g. atom of Fe per litre, and its purity was 2.06.

TABLE III.

Sol concentration = 0.268 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c. of which 9 c.c. contain 0.000126 g. atom of Fe.

Amount of electrolyte.	Volume of coagulum.	Amount of precipitate peptised.
N/2-KCl.		Nil
5.0 c.c.	0.5 c.c.	0.000029 g. atom
4.0	0.5	0.000031
3.0	0.5	
2.5	Partial coagulation	
N/40-K ₂ SO ₄ .		
2.5 c.c. to		
1.0 c.c.	0.5 c.c.	Nil
0.5	No coagulation	

With this concentration and purity of the sol, numerous thick rings appeared (Plate I) on complete coagulation with lower concentrations of KCl. Higher concentration of KCl coagulated the sol more rapidly and only broken bands appeared there. There was a tendency for the formation of bands with K₂SO₄ as coagulant. Higher concentration of the sol yielded coagulum in gel state with no settling in 24 hours. With lower concentration of the sol rings appeared but all were broken due to complete settling of the coagula.

The speed of coagulation of this sol with KCl at its precipitating concentration is of the same nature as in Table II. The speed with K_2SO_4 could not be followed as the coagulation with this electrolyte was almost instantaneous.

The original sol was further purified by hot dialysis for 3 hours and contained 0.40 g. atom of Fe per litre, and its purity was 6.66.

TABLE IV.

Sol concentration = 0.04 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c.

Amount of $N/2-KCl$	Volume of coagulum	
5 c.c.	0.7 c.c.	
4	0.7	
3	0.75	Nothing peptised
2	Partial coagulation	

With this concentration of the sol, the coagula were all in the gel state and did not settle at all when coagulated by KCl or K_2SO_4 .

TABLE V.

Sol concentration = 0.02 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c. of which 9 c.c. contain 0.0009 g. atom of Fe.

Amount of $N/2-KCl$	Volume of coagulum	
5 c.c.	0.6 c.c.	Nothing peptised
4	0.6	Do
3	Partial coagulation	

With this concentration of the sol, the coagula were all in the gel state as before after 24 hours. After 48 hours, the coagula settled slightly and a few rings appeared. With still lower concentration of the sol, the coagula settled slightly but the amount of the coagula was so small that no bands developed fully.

Ferric Hydroxide Sol (Carbonate Method).

The sol was prepared by adding a solution of ammonium carbonate to ferric chloride solution in stages and with frequent stirring until the precipitate thus formed was peptised with difficulty. The resulting sol was purified by cold dialysis for 3 days and contained 0.142 g. atom of Fe per litre and of purity of 0.12.

TABLE VI.

Sol concentration = 0.0284 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c.

Amount of $N/40\text{-K}_2\text{SO}_4$.	Volume of coagulum	Amount of precipitate peptised
5 to 3 c.c.	0.35 c.c.	Nil
2 c.c.	Partial coagulation	

With this concentration of the sol, there was a tendency for the formation of rings but as the coagulation with K_2SO_4 was almost instantaneous the bands could not develop. The sol could not be coagulated with KCl.

TABLE VII.

Speed of coagulation

8 C.c. of $N/40\text{-K}_2\text{SO}_4$ (made up to 10 c.c.) coagulated 10 c.c. of the sol almost instantaneously.

Time	Amount left in sol state out of 0.000295 g. atom of Fe
After 2 min	0.00048 g. atom
15	0.00032
30	0.00024

The original sol was further purified by cold dialysis for 4 days and contained 0.1396 g. atom of Fe per litre and its purity was 4.93.

TABLE VIII.

Sol concentration = 0.02792 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c.

Amount of $N/2\text{-KCl}$	Volume of coagulum.	Amount of precipitate peptised.
2.5 c.c.	0.35 c.c.	Nil
2.0	0.40	Negligible
1.5	0.45	0.000018 g. atom
1.0	0.50	0.000054
0.5	Partial coagulation	

With this concentration of the sol, quite prominent rings developed (Plate II) with lower amounts of KCl, *i.e.*, with 1.5 and 1.0 c.c.; with greater amounts of the electrolyte, broken rings appeared as the coagula settled more or less completely. K_2SO_4 coagulated the sol almost instantaneously producing no rings. Higher concentrations of the sol yielded coagula in gel state and no rings developed, and with lower concentration of the sol the coagula settled completely and the few rings which developed were all broken.

TABLE IX.

Speed of coagulation

7 C.c. of $N/2$ -KCl made upto 10 c.c. coagulated 10 c.c. of the sol in 1 hour (precipitating concentration).

Time	Amount left in sol state out of 0.002295 g. atom of Fe
After 2 min.	No coagulation
15	0.000283 g. atom
45	0.000116
60	0.000058

Adsorption of Sol.

The adsorption experiments were performed as stated in the previous paper (*loc. cit.*)

TABLE X.

Amount of precipitate taken each time = 0.35 g. of Fe. Amount of sol taken each time = 0.1262 g. of Fe.

Amount of $2N$ -KCl.	Amount of Fe left in sol state.	% Adsorption.
0 c. c.	0.134	-6.18
1	0.0186	84.90
2	0.000	100.00

In the absence of the precipitate, the same amount of the sol required 6.5 c. c. of the electrolyte for partial and 10.5 c.c. for complete coagulation.

The original sol was further purified by cold dialysis for 6 days. It contained 0.138 g. atom of Fe per litre and its purity was 8.62.

TABLE XI.

Sol concentration = 0.0276 g. atom of Fe per litre. Sol taken each time = 10 c.c. Total volume = 20 c.c. of which 9 c.c. contain 0.0001242 g. atom of Fe.

Amount of N/4-KCl.	Volume of coagulum.	Amount of precipitate peptised.
5 c. c.	0.40 c. c.	Nil
4	0.45	"
3	0.45	"
2	0.50	"
1	Partial coagulation	

With this concentration and purity of the sol, quite prominent rings appeared with lower concentration of KCl, but the number of rings was less than those obtained with the previous sol. K_2SO_4 coagulated the sol almost instantaneously, producing no rings.

TABLE XII.

Speed of coagulation.

1.5 C.c. of the sol made up to 10 c.c. coagulated 10 c.c. of the sol in 1 hour. (precipitating concentration).

Time.	Amount left in sol state out of 0.000295 g. atom of Fe.
After 2 min.	0.000270 g. atom
15	0.000185
45	0.000075
60	0.000044

TABLE XIII.

Adsorption of sol.

Amount of precipitate taken each time = 0.14 g. of Fe. Amount of the sol taken each time = 0.07 g. of Fe.

Amount of N/4-KCl.	Amount of Fe left in sol state	% Adsorption.
0	0.078 g.	11.4
1 c c	0.061	12.6
2	0.016	78.5
3	0.009	100.0

In the absence of the precipitate, the same amount of the sol required 8.0 c.c. of the electrolyte for partial and 10.2 c.c. for complete coagulation.

Ferric Hydroxide Sol (Kiecke's Method).

The sol was prepared by adding a saturated solution of ferric chloride to boiling water drop by drop. The resulting sol was purified by hot dialysis for 50 hours. It contained 0.07378 g. atom of Fe per litre and its purity was 21.7.

TABLE XIV.

Sol concentration = 0.07378 g. atom of Fe per litre. Amount of sol taken each time = 10 c.c. Total volume = 20 c.c. of which 9 c.c. contain 0.0003332 g. atom of Fe.

Amount of $N/2$ -KCl.	Volume of coagulum	Amount of precipitate peptised.
6 c. c.	0.20 c. c.	0.000042 g. atom
5	0.15	0.000048
4	0.15	0.000078
3	Partial coagulation	

With this concentration of the sol, no rings appeared with KCl or K_2SO_4 . There was, however, a tendency for the formation of thin bands with KCl. The coagulum was of typically lyophobic nature.

TABLE XV.

Speed of coagulation.

4 C.c. of $N/20$ -KCl (made up to 10 c.c.) coagulated 10 c.c. of the sol almost instantaneously (ppt. conc.).

Time.	Amount left in sol state out of 0.000295 g. atom of Fe.
After 2 min	0.000044 g. atom
15	0.000032
30	0.000029
60	0.000029

TABLE XVI.
Adsorption of sol.

Amount of precipitate taken each time = 0.14 g. of Fe. Amount of the sol taken each time = 0.02 g. of Fe.

Amount of N/2-KCl.	Amount of Fe left in sol state	% Adsorption.
0 c. c.	0.022	6.8
1	0.003	85.4
2	0.000	100.0

In the absence of the precipitate, the same amount of the sol required 11.5 c.c. of the electrolyte for partial and 15.5 c.c. for complete coagulation.

The original sol was further purified by hot dialysis for 150 hours. It contained 0.082 g. atom of Fe per litre and its purity was 120.6. Though this sol was fairly pure, it behaved just as previously. The nature of the coagulum did not improve and no rings were obtained with it.

DISCUSSION.

From the foregoing results it may be seen that moderately pure sol of ferric hydroxide, prepared either by acetate or carbonate method, produces periodic precipitates, when coagulated by univalent electrolyte like KCl but the same sol prepared by Krecke's method does not produce such periodicities, irrespective of the nature purity and concentration of the sol taken. It is well known that the properties of the same sol, like that of ferric hydroxide, prepared by different methods vary widely according to the method of preparation adopted. The colloidal micelle from a sol prepared by acetate and carbonate methods are moderately hydrated. This can be judged from the data on the volumes of the coagula yielded by different sols on coagulation which are remarkably more hydrated in the case of the sols prepared by acetate and carbonate methods. It has been generally noted that an optimum volume of the coagula, which settle periodically, obtained from 9 c.c. out of the total volume of 20 c.c. of the sol-electrolyte mixture, has been 0.5 c.c. Values above and below this optimum volume are not favourable for the process of ring formation. It has also been found that there are two conditions for greater or less values of the volumes of coagula obtained from a definite amount of Fe_2O_3 in colloidal condition. The one is that if the coagulation of the sol be effected rapidly for a certain purity of it, the volume of coagulum becomes less. For with rapid coagulation, the colloidal particles are discharged completely and the precipitate is of a compact mass. On the other hand, if the sol be made purer by dialysis, the speed of

coagulation becomes rapid but as the colloid becomes more hydrated the coagulum occupies greater volume.

From the data on the speed of coagulation it is seen that the coagulation of sol with K_2SO_4 is practically complete within two minutes after the addition of the electrolyte (*cf.* Tables II, VII). The coagulation being rapid, this electrolyte is not suitable for producing rings. With KCl the coagulation is slow near about its precipitating concentration and sufficient coagulation takes place fifteen minutes after the addition of the electrolyte (*cf.* Tables II and IV). This electrolyte is suitable for producing rings, provided the optimum volume of the coagulum be 0.5 c.c under the conditions as stated above. With increasing amounts of KCl, the speed of coagulation increases (*cf.* Tables II, XII) and both the qualities and the number of rings fall. In the case of the ferric hydroxide sol prepared by Krecke's method, the speed of coagulation with KCl is rapid and is of the same nature as in Tables II and VII and this sol does produce periodicities as in other cases. During the process of centrifuging, specially in the cases of slow coagulation, it was observed that some of the precipitate got peptised and the amount of the precipitate thus peptised increases with the lesser amounts of the electrolyte; because in the latter case the speed being slow, the coagulum already appeared coexists with its own sol and adsorbs the uncoagulated sol. This loosely adsorbed sol comes out as the coagulum becomes more and more compact during centrifuging. This adsorption of sol can explain the S-shaped nature of some of the coagulation velocity data (Tables II and IX). The coagulation proceeds at first slowly, then rises quickly as the coagulum appears more and more and finally falls off again. In these cases quite good rings appear. With the increasing amounts of the coagulating electrolyte, the amount of the peptised sol during centrifuging decreases and no good rings appear here due to small adsorption of sol by the coagulum.

Thus the adsorption of sol by its own precipitate is an important factor in the formation of rings. But it has been observed that the adsorption is greatest in the case of the ferric hydroxide sol prepared by Krecke's method where no good rings are obtained. In the sols prepared by acetate and carbonate methods the adsorption is also appreciable. It may be concluded, therefore, that the adsorption of sol by its own precipitate is fairly general in colloids and is not a guiding factor in the process of the formation of rings in sols by coagulation, as are the other two factors viz. (i) the nature of the coagulum and (ii) the speed of the coagulation.

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Received February 6, 1939.

CUPRIC HYDROXIDE SOL.

By R. N. MITTRA.

Cupric hydroxide sol has been prepared and its various properties with coagulation, abnormal dilution effect, ionic antagonism etc. have been investigated.

Very little work has been done on the preparation of cupric hydroxide sol in the absence of protective agents like cane sugar (Sen and Dhar, *Kolloid Z.*, 1923, **33**, 193), grape sugar, casein, sodium salts of protalbinic and lysalbinic acids etc. which have been found to stabilise cupric hydroxide sols. Kohlshutter (*Z. Electrochem.*, 1919 **25**, 309) reported the formation of a sol when a current of high density was passed between copper electrodes in dilute copper sulphate solution. Burton (*Phil. Mag.*, 1906, **11**, 436) prepared a positively charged unstable cupric hydroxide sol by passing a spark between copper electrodes under water. Hooker (*Ind. Eng. Chem.*, 1923, **15**, 1177) obtained a blue sol by washing the gelatinous precipitate of cupric hydroxide formed by adding alkali to copper sulphate solution.

EXPERIMENTAL.

In an attempt to prepare a pure sol of cupric hydroxide, it was found that copper sulphate is not a satisfactory starting material because of the coagulating effect of the sulphate ions on the sol formed. Cupric chloride was, however, found to be a suitable starting material for this sol. A dilute solution of ammonium hydroxide was added very gradually to a solution of cupric chloride when the amount was just sufficient to throw down a gelatinous precipitate of cupric hydroxide. The precipitate was allowed to settle, the supernatant liquid decanted off and finally the compact precipitate was peptised with minimum quantity of water, when a dirty blue sol resulted. The undialysed sol was taken for investigation. It was further purified by cold dialysis to different extents for further studies. The following are the results obtained.

TABLE I.

Concentration and purity.

	Sol I. (Undialysed)	Sol II. (Dialysed for 48 hrs)	Sol III. (Dialysed for 96 hrs.)
CuO (g./litre)	3.26	2.54	1.98
Chloride „	3.41	1.93	1.59
Purity	0.448	0.62	0.60

TABLE II.

Coagulation.

The sols were maintained at the same concentration *i.e.*, 1.63 g. of CuO per litre. Sol taken each time = 5 c.c. Total volume = 10 c.c. Time for complete coagulation = 1 hour

Sol I				
Sol conc	Precipitating concentration			Ratio KCl
	KCl	NH ₄ Cl	K ₂ SO ₄	$\frac{KCl}{K_2SO_4}$
Sol I	0.165M	0.190M	0.000425M	388
Sol I/2	0.180	0.220	0.000275	655
Sol I/4	0.195	0.250	0.000175	1114
Sol II				
Sol II	0.225		0.00035	620
Sol II/2	0.235		0.00022	1068
Sol II/4	0.245		0.00017	1441
Sol III.				
Sol III	0.220	...	0.00022	1000
Sol III/2	0.227	..	0.00016	1419
Sol III/4	0.235	...	0.00012	1958

TABLE III.

• *Coagulation with mixture of KCl and K₂SO₄.*

N/200-K₂SO₄ for complete coagulation.

Sol I.						
N/2-KCl (c.c.)	0	3.3	1.0	2.0	3.0	
Obs.	...	1.7	0	1.8	1.5	0.7
Calc.	1.19	0.67	0.15
Diff.	.		0.61	0.83		0.55

TABLE III (*contd.*).

Sol II.						
N/2-KCl (c.c.)	0	4.5	1.0	2.0	3.0	
Obs.	...	1.4	0	1.5	1.3	0.9
Calc.	1.09	0.77		0.47
Diff.	0.41	0.53		0.43
Sol III.						
N/2-KCl (c.c.)	...	0	4.4	1.0	2.0	3.0
Obs.	...	0.9	0	1.0	0.9	0.6
Calc.	0.7	0.5	0.29
Diff.	0.3	0.4	0.31

TABLE IV.

Viscosity of the sol at 35°.

	Viscosity.	Density
Sol I	1.06	1.021
Sol II	1.04	1.015
Sol III	1.03	1.009
Water	1.00	...

A fresh sample of the sol was prepared and subjected to hot dialysis to different extents. The following were the results obtained.

TABLE V.

Concentration and purity.

	Sol A. (Dialysed hot for 50 hours).	Sol B. (Dialysed hot for 90 hours.)
CuO (g./litre)	...	7.34
Chloride (,,)	...	2.59
Purity	...	1.39
		6.99
		1.98
		1.66

TABLE VI

Coagulation.

The sols were maintained at the same concentration of 1.63 g of CuO per litre. Sol taken each time = 5 c.c. Total volume = 10 c.c. Time for complete coagulation = 1 hour.

		Sol A			
Sol conc		Precipitating concentrations			
		KCl	KBrO ₃	KIO ₃	K ₂ SO ₄
Sol A	...	0.11M	0.023M	0.0028M	0.00032M
A/2	...	0.12	0.023	0.0028	0.00019
A/4	...	0.13	0.025	0.0028	...
		Sol B			
Sol B	...	0.02M	0.019M	0.0024M	0.00029M
B/2	...	0.021	0.020	0.0024	...
B/4	...	0.023	0.022	0.0024	...
		Ratios of ppt. conc			
		KCl	KBrO ₃	KIO ₃	K ₂ SO ₄
Sol A	...	344	72	9	1
Sol B	...	67	65	8	1

TABLE VII.

Coagulation with mixture of KCl and K₂SO₄.

N/400-K₂SO₄ for complete coagulation.

		Sol A.			Sol B				
N/2-KCl (c c)		0	2.2	1.0	1.5	0	4.0	2.0	3.0
Obs.	...	2.6	0	1.3	0.7	2.3	0	1.22	0.55
Calc	1.4	0.8	1.15	0.58
Diff.	0.1	0.1	.	..	0.07	0.03

TABLE VIII.

Viscosity of the sols at 35°.

Sol.			Viscosity.	Density
Sol A	1.06	1.04
Sol B	1.05	1.03
Water	1.00	—

DISCUSSION.

From the foregoing results it may be seen that cupric hydroxide sol could not be obtained in so highly concentrated condition as the sols previously studied (Mitra and Dhar, *J. Indian Chem. Soc.*, 1932, 9, 315). To whatever lengths of time the sols were dialysed for purification, they could not be freed completely from their respective stabilising ions. This high adsorbability of the stabilising ions did not allow the development of lyophilic character of the sols, for the viscosities of the sols were not different from the typically lyophobic ones. It must be emphasised here that in sols behaving in a lyophilic manner like those of ferric hydroxide, aluminium hydroxide, etc., the higher the hydration, the less is the charge on the colloid particles and conversely higher charge on the colloid particles is responsible for low viscosity of the sol. It has been shown (Dhar and Mitra, *Kolloid Z.*, 1935, 71, 172) that by adding thorium nitrate to a purified sol of thorium hydroxide of a high viscosity, it became less viscous. From the precipitating concentrations of the electrolytes and their ratios for different valencies it may also be seen that the sols were very impure, and the purity data supported that.

It may be observed here that the amounts of a monovalent ion, such as chloride, required to coagulate the sols of cupric hydroxide increased with the progressive dialysis of the sols and finally decreased. Similarly the ratios of the precipitating concentrations, mono: bi: tri: quadri-valent ions had tendency to decrease (Table VI). It is a general experience that the sols, prepared by the interaction of two or more substances in solutions, producing electrolytes as resultants, should immediately be dialysed as soon as they are prepared, for the electrolytes present in the sols have a coagulating influence, though it is a very slow process. It is necessary at this stage to mention the results of Desai and co-workers (*J. Indian Chem. Soc.*, 1932, 9, 463) on the stability of gold and ferric hydroxide sols on dialysis. These authors report that in general, the electric charge on colloid particles increases, passes through a maximum and then quickly falls with the progressive dialysis.

The experimental results on the coagulation of cupric hydroxide sols of different concentrations and degrees of purity, prepared by hot and cold dialysis, indicate that greater quantities of potassium chloride or ammonium chloride are necessary to coagulate a dilute sol than a concentrated one, and this abnormal behaviour of dilution towards potassium chloride is more prominent with impure sols than with purer ones. The sol prepared by hot dialysis also develops this phenomenon though less prominently. It has been reported (Ghosh and Mitra, *J. Indian Chem. Soc.*, 1933, 10, 471) that

change of stability of sols of various concentrations is ultimately related with their purity, besides the effect of the similarly charged ions as has been emphasised by Ghosh and Dhar (*J. Phys. Chem.*, 1927, **31**, 647). It has been shown by the author that sols of ferric, aluminium, chromium (*cf.* Mittra and Dhar, *loc. cit.*) and thorium hydroxides (*cf.* Dhar and Mittra, *loc. cit.*) are stable towards electrolytes like potassium chloride on dilution, when large quantities of stabilising electrolytes are present.

It has already been pointed out that in the cases of impure sols, the abnormal behaviour on dilution towards its coagulation by monovalent electrolytes develops in the presence of large amounts of stabilising electrolytes but the additive relationship for the electrolytes of different valencies is maintained, provided the sol is incapable of adsorbing the similarly charged ions of the added electrolytes. The results in Table III show that amounts of potassium sulphate greater than the calculated amounts are necessary to coagulate a cupric hydroxide sol, purified by cold dialysis in the presence of potassium chloride; in other words, ionic antagonism is observed in coagulating this sol by a mixture of potassium chloride and potassium sulphate. In order to see how far the adsorption of similarly charged ions is responsible for such behaviour of cupric hydroxide sol, the following experiments were performed on the adsorption of ammonium and chloride ions from ammonium chloride by different amounts of copper hydroxide.

A definite volume (50 c. c.) of the sol was taken in separate graduated 100 c. c. flasks. The sols were coagulated by definite amounts of potassium sulphate. To one of the flasks 10 c. c. of the standardised ammonium chloride solution (5 c. c. of which contained 0.145 g. of ammonium chloride) and to the other 20 c. c. of it were added, while the third one was kept without it. All the three were made up to 100 c. c. by water and kept overnight. The supernatant clear liquid (5 c. c.) was carefully pipetted out from each of the three flasks and heated on water-baths with excess of standard potassium hydroxide solution. The unacted potassium hydroxide was titrated back with standard hydrochloric acid in each case, from which the ammonium equivalents were determined. The amounts of ammonium ions, already present while preparing the sol, was determined from the third flask with no extra ammonium chloride and subtracted from the total amount of ammonium ions from each of the other two flasks. Similarly the amounts of the chloride ions unadsorbed from the added ammonium chloride were estimated from the first two flasks.

Similar experiments were repeated by taking 25 c. c. of the sol, keeping the other conditions the same.

TABLE IX.

	%Adsorption with 10 c.c. of NH_4Cl		%Adsorption with 20 c.c. NH_4Cl .	
	NH_4 .	Cl	NH_4 .	Cl.
Sol (50 c.c.)	17.2	13.4	35.0	11.54
Sol (25 c.c.)	8.0	4.6	11.4	1.80

The above experiment clearly proves that cupric hydroxide can adsorb appreciable amounts of cations like NH_4 from NH_4Cl solution. It will be also noted here that the adsorption of Cl ions is small in comparison to NH_4 ions and further the ratio of the percentage of adsorption of NH_4 ions to that of Cl ions increases with the decreasing amount of the adsorbent. These results confirm the views of Ghosh and Dhar (*loc cit*) on the abnormal behaviour of sols on dilution in the cases where the ions, carrying the same charge as the colloid particles, from a coagulating electrolyte, play an important part. The above experimental results, therefore, prove that the stability of cupric hydroxide sol on dilution towards its coagulation by ammonium chloride is partly due to the adsorption of the similarly charged cation from ammonium chloride and partly due to the large amount of the stabilising electrolyte present in the sol.

Ghosh and Dhar have concluded that for the sols which are capable of adsorbing the ions carrying the same charge as the colloid particles from a coagulating electrolyte, abnormal dilution effect, ionic antagonism and the phenomenon of acclimatisation are developed. The following results were obtained by the present author on the phenomenon of acclimatisation of cupric hydroxide sols dialysed in the cold when coagulated by NH_4Cl .

TABLE X.

Time allowed for the coagulation to proceed = 24 hours.

	Amount of N/2-KCl added	
	all at once	by parts.
Sol I	2.25 c.c.	5.0 c.c.
Sol II	3.1	4.5
Sol III	3.0	4.2

The results presented in Tables II and III show that the abnormal dilution effect for the coagulation of cupric hydroxide sol by KCl and the ionic antagonism for a mixture of KCl and K_2SO_4 for this sol become less

prominent when the sol is dialysed to greater extents. Similarly the results in Tables VI and VII show that both the abnormal dilution effect and ionic antagonism are not so prominent in the sol prepared by hot dialysis as observed with the impure sample of cupric hydroxide sol obtained by cold dialysis. Also the capability for adsorption of cations like K or NH_4 by cupric hydroxide greatly diminishes with ageing, which is considerably hastened by the process of heating.

It may be of interest to discuss here some of the results of Sorum and Judd (*J. Amer. Chem. Soc.* 1930, **52**, 2591) on the stability of ferric hydroxide sol. These authors report that the Burton-Bishop rule or so-called abnormal dilution effect of Ghosh and Dhar is observable with this sol when it is purified to its extreme extent. My results on the coagulation of ferric hydroxide sol prepared by acetate method lead to conclusions opposed to those of Sorum and Judd. It has been found that ferric hydroxide sol of purity 63.28 showed abnormal dilution effect towards its coagulation with electrolytes, while the sol which was highly pure and whose purity could not be determined showed normal effect.

In the opinion of the present author if a sol is not sufficiently pure and contains large amounts of the stabilising electrolytes, the sol may behave abnormally towards the coagulation by univalent electrolytes on dilution, in spite of the fact that the sol is incapable of adsorbing similarly charged ions from the coagulating electrolytes leading to the additive relationship when the sol is coagulated by a mixture of electrolytes.

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Received February 6, 1939.

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FIG. 1



FIG. 2



NATURAL FLAVONES. PART III. ON THE CONSTITUTION OF TAMBULIN.

BY PRAFULLA KUMAR BOSE AND JOGENDRALAL BOSE.

From the fruits of *Zanthoxylum acanthopodium* DC., two yellow crystalline substances have been isolated in poor yields. One of these, named tambulin, $C_{18}H_{16}O_7$, has been shown to be a dihydroxytrimethoxyflavone. Alkaline hydrolysis of tambulin gives anisic acid. Dimethyltambulin is not identical with tangeretin and tambulin is either 5 : 7-dihydroxy-3 : 8 : 4'-trimethoxyflavone or 5 : 7-dihydroxy-4' : 6 : 8-trimethoxyflavone.

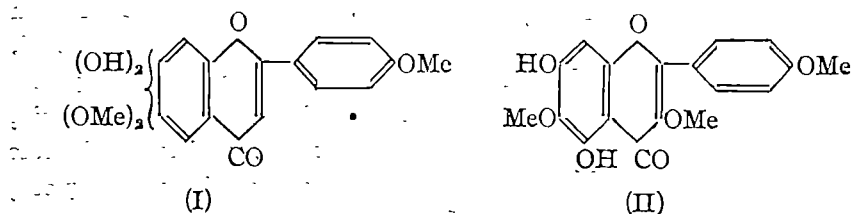
Zanthoxylum acanthopodium DC. (N.O. *Rutaceae*) is a well known indigenous drug of the East. In Bengal the fruits are known as *Tambul*. According to Ayurveda 'the fruit is sweetish, bitter, hot; tasty and digestible; appetiser; anthelmintic; removes *kapha* and *vata*, pain, tumours and abdominal troubles; useful in eye and ear diseases, diseases of the lips, headache, heaviness, leucoderma, asthma, troubles of the spleen, difficult micturation.' According to the Yunani system of medicine, 'the seeds are tonic, useful in diarrhoea; carminative, bechic, pectoral; good in brain diseases and insanity; useful in stomatitis; strengthen the liver; purify the blood; remove foul smell from mouth.' In Indo-China the aromatic seeds are considered sudbrific and febrifuge.

The essential oil, which is present to the extent of 1.2 per cent, has been the subject of an investigation by Simonsen and Rau (*Indian Forest Records*, 1922, 9, 111). The chief constituents of the oil are dipentene, methyl cinnamate and linalool. Other constituents of the fruits do not seem to have been investigated. The present paper deals with two crystalline colouring matters of the fruits, which have been isolated. These have been named tambulin and tambulol.

Tambulin (yield 0.006 %) forms bright yellow plates, m.p. 265° . It is easily soluble in cold dilute aqueous alkali with a deep yellow colour, and the alkaline solution is stable to aerial oxidation. Ferric chloride imparts a dark olive-green colour to an alcoholic solution of tambulin. Evidently it is phenolic in character. The pale yellow solution of tambulin in absolute alcohol turns orange on the addition of a drop of concentrated aqueous lead acetate solution, and an orange precipitate is thrown down on dilution with water. Alkaline *o*-dinitrobenzene (Bose, *J. Indian Chem. Soc.*, Sir P. C. Ray 70th Birthday Volume, p. 65) or chloropentammine cobaltichloride (Shibata and Hattori, *Acta Phytochim.*, 1930, 5, 117) have no action on tambulin. It dissolves in hot concentrated hydrochloric acid

with a yellow colour, evidently due to the formation of an oxonium salt. If this solution be diluted with alcohol and treated with metallic magnesium, a beautiful rose-red colour is immediately produced. These properties indicate that tambulin is a hydroxyflavone.

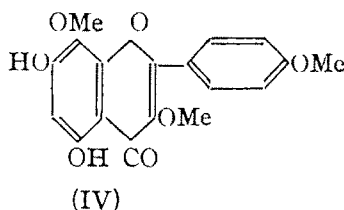
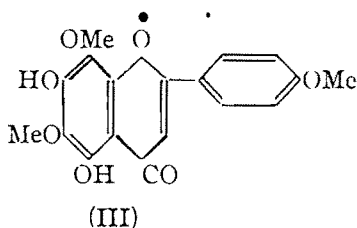
Tambulin contains methoxy groups, and the analytical data are in agreement with the formula, $C_{15}H_5O_2(OH)_2(OMe)_3$. It gives the expected diacetyl derivative. Hydrolysis of tambulin with 20% alcoholic potassium hydroxide gives products from which a colourless crystalline acid, m.p. $179-80^\circ$, has been isolated. Its analytical data point to the formula, $C_8H_4(OMe)CO_2H$ and it has been identified as anisic acid. The partial formula for tambulin may, therefore, be written as (I).



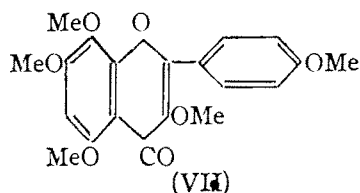
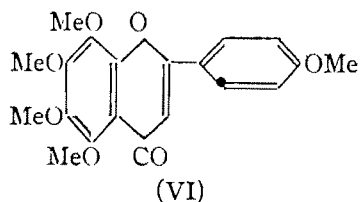
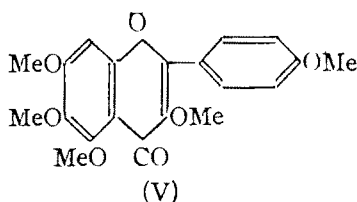
The other products of hydrolysis could not be isolated as sufficient material was not available for hydrolysis. Since one of the methoxy groups is present in the phenyl nucleus, the two OH groups as also the remaining two OMe groups must be in the benzopyrone nucleus. One of the OH groups may be assumed to be in position 5, since in partially methylated polyhydroxyflavones, the OH group in position 5 is not, as a rule, found in the methylated condition.* The second OH group cannot be in position 3, owing to the stability of an alkaline solution of tambulin to aerial oxidation. This OH group must be in position 7, because, otherwise tambulin would have a structure having OH groups in *ortho*- or *para*-positions to each other, and would consequently show reducing action towards *o*-dinitrobenzene and chloropentamine cobaltichloride, which was not found to be the case (*vide supra*). Hence three alternative formulæ for tambulin are possible, namely (II), (III) and (IV). The demethylated products from (II) and (III) should give a positive Bargellini's test, which is characteristic of three contiguous OH groups in positions 5, 6 and 7 of a benzopyrone nucleus (Bargellini, *Gazzetta*, 1919, **49**, 47;

* The only exception appears to be calycopterin (=thapsin), in which, according to the suggestion made by Mahal and Venkataraman (*Current Sci.*, 1935, **4**, 311), the 5 position carries a methoxyl group and the 6 position a hydroxyl. Further work has shown that the suggested structure must be confirmed by additional evidence which is now being sought (Venkataraman, *private communication*).

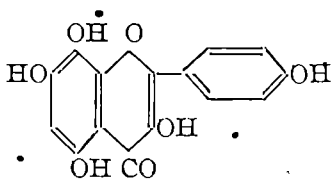
Baker, *J. Chem. Soc.*, 1938, 1030; Baker, Nodzu and Robinson, *ibid.*, 1929, 83).



Tambulin on demethylation by means of hydriodic acid gives a product, an alcoholic solution of which yields a bluish green flocculent precipitate with freshly prepared sodium amalgam. This positive Bargellini's test can be expected from the demethylation products of both (II) and (III). If the formula (II) represent the correct structure of tambulin, then its dimethyl ether should be identical with tangeretin (Nelson, *J. Amer. Chem. Soc.*, 1934, **56**, 1392; Goldsworthy and Robinson, *J. Chem. Soc.*, 1937, 46). To settle this point we methylated tambulin with an excess of diazomethane. The ether, purified by distillation in high vacuum and crystallisation, melts at 160° (uncorr.), whereas the recorded m.p. of tangeretin is 154° (corr.). Moreover, demethylated tangeretin develops a green colour quickly, which is not found to be the case with the demethylated product of tambulin. These differences in m.p. and colour suggest that dimethyltambulin is different from tangeretin. A direct comparison of these two substances has, moreover, been possible, through the kindness of Dr. E. K. Nelson, to whom our best thanks are due. Although the outward appearances of the compounds are very similar, a mixture of the two melted between 146° and 151°. The formula (II) for tambulin had consequently to be rejected.



The formula (IV) has already been left out of consideration, since its demethylated product should not be expected to give a positive Bargellini's test. We, however, wanted to confirm this point. Quite recently the compound (VIII) has been isolated in the form of a glucoside, herbacitrin, from the flowers of certain cotton plants (Neelkantam and Seshadri, *Proc. Indian Acad. Sci.*, 1937, **A**, **5**, 357) and the aglucone (VIII) has been synthesised by Goldsworthy and Robinson (*J. Chem. Soc.*, 1938 56). Prof. T. R. Seshadri kindly carried out Bargellini's test with herbacetin at our request, and informed us that the test was positive, the colour of the precipitate being bluish green (*cf.* Rao and Seshadri, *Current Sci.*, 1938, **7**, 228). This was rather unexpected, since a positive Bargellini's test has always been associated with 5:6:7-trihydroxybenzopyrones (*vide supra*). The observation of Prof. Seshadri, which is very significant, shows that a herbacetin type of formula for tambulin, namely (IV), is not excluded. As a matter of fact the difference in m.p. of herbacetin pentamethyl ether and tambulin dimethyl ether is only 2–4°. A direct comparison of these two substances has not yet been possible for want of an authentic specimen of herbacetin pentamethyl ether.



(VIII)

EXPERIMENTAL.

Isolation of Tambulin.—The fruits of *Zanthoxylum acanthopodium* DC. were supplied by a local dealer in drugs and were identified by Mr. N. Mitra, Curator, Royal Botanic Garden, Sibpur, to whom our best thanks are due. 500 G. of coarsely powdered fruits were refluxed with a litre of rectified spirit for 2 hours and filtered hot. The process of extraction was repeated four times, using the same volume of fresh solvent in each operation. The combined filtrates which had a brown yellow colour, were concentrated on the water-bath under reduced pressure. The brown oily residue was then poured into 500 c.c. of petroleum ether, when a tar separated out. The petroleum ether layer (A) was separated from the tar (B) by decantation and allowed to stand in a closed vessel for several weeks at the room temperature. The tar (B) was dissolved in rectified spirit and allowed to stand.

Small yellow crystals gradually separated out from Fraction A and were collected after 4 weeks. The crude product was washed with a small quantity of cold rectified spirit to remove tarry matters and then dissolved in chloroform, in which it freely dissolves. The chloroform solution was filtered and the solvent completely removed. The bright yellow crystalline residue was twice crystallised from acetone and then thrice from glacial acetic acid, when deep yellow plates, m.p. 205° , were obtained. Further crystallisations did not raise the m.p. The yield was 0.75 g. from 12 kg. of fruits. (Found: C, 62.29, 62.20; H, 4.88, 4.73; OMe, 26.44. $C_{18}H_{16}O_7$ requires C, 62.79; H, 4.65; OMe, 27.04 per cent).

Tambulin dissolves freely in chloroform, benzene, acetone and hot glacial acetic acid; it is sparingly soluble in alcohol and ethyl acetate, and insoluble in petroleum ether.

Isolation of Tambulol.—From the alcoholic solution of (B), a small quantity of another yellow compound separated out gradually. This was collected, washed with rectified spirit and then repeatedly crystallised from aqueous pyridine, till the m.p. became constant at $265-67^{\circ}$. This substance, which has been named tambulol, is insoluble in most organic solvents. The yield is 0.2 g. from 12 kg. of fruits. (Found in samples dried at $140-45^{\circ}$ in *vacuo* over P_2O_5 : Sample I gave C, 52.95; H, 4.64. Sample II gave C, 52.01; H, 4.39 per cent). Tambulol is soluble in aqueous alkali and the alkaline solution has no reducing action on *o*-dinitrobenzene. Concentrated hydrochloric acid dissolves it in the cold forming an orange solution, which does not deepen on treatment with alcohol and metallic magnesium. The acetyl derivative, prepared in the usual manner, crystallised from ether-petroleum ether in flakes, m.p. 120° (not sharp).

Acetyltambulin.—0.1 G. of tambulin, 3 c.c. of acetic anhydride and a drop of pyridine were refluxed for 3 hours. The product was cooled, diluted with 20 c.c. of ether and filtered. The filtrate was freed from ether, acetic anhydride, etc., in vacuum and the brown residue was taken up in benzene, charcoaled, concentrated and carefully diluted with petroleum ether. The colourless crystals were collected next day and washed with petroleum ether, m.p. $160-61^{\circ}$. (Found: C, 61.31; H, 4.60; OMe, 21.19. $C_{22}H_{20}O_6$ requires C, 61.69; H, 4.67; OMe, 21.73 per cent).

Hydrolysis of Tambulin.—Tambulin (0.2 g.) was refluxed on the water-bath for 10 hours with alcoholic potash (20 %, 10 c.c.). The red solution was freed from alcohol under diminished pressure and the residue taken up with about 10 c.c. of water. The solution was then saturated with carbon dioxide at 0° when a yellow slimy precipitate separated out. This was filtered off and the clear filtrate was twice shaken up with small quantities of ether.

The brown-red solution was then concentrated on the water-bath and then acidified with hydrochloric acid. The greyish white precipitate was collected, washed with a little ice-cold water, dried and then sublimed in vacuum at $110-20^{\circ}/0.05$ mm. The sublimate formed long colourless needles, m.p. $179-80^{\circ}$, from dilute alcohol. (Found: C, 62.90; H, 5.35; OMe, 19.75. Calc. for $C_8H_8O_3$: C, 63.17; H, 5.26; OMe, 20.40 per cent). Mixed with a specimen of anisic acid, m.p. $180-81^{\circ}$, there was hardly any depression of m.p. The physical properties of both the compounds were similar. It is therefore anisic acid.

Dimethyltambulin (Tambulin Dimethyl Ether).—Tambulin (0.1 g.) dissolved in dry pure methyl alcohol (20 c.c.) was cooled in ice. To this was added a solution of diazomethane in ether (nearly double the calculated quantity) and the mixture was allowed to stand for 2 days at room temperature. Solvents were then distilled off from the yellow solution and the residue crystallised from dilute methyl alcohol in cream-coloured needles, m.p. $154-55^{\circ}$. It was then distilled in high vacuum ($210-20^{\circ}/0.05$ mm.) and crystallised from ethyl acetate-petroleum ether as cream-coloured prisms, m.p. 160° . (Found: C, 64.55; H, 5.60; OMe, 41.42. $C_{20}H_{20}O_7$ requires C, 64.52; H, 5.38; OMe, 41.67 per cent). The ether is insoluble in cold aqueous alkali and does not give any colour with ferric chloride in alcohol. Concentrated nitric acid produces a blood-red colour.

Demethylation of Tambulin.—Tambulin (0.05 g.) was dissolved in the minimum quantity of hot phenol and then mixed with hydriodic acid (d 1.7; 5 c.c.). The mixture was heated for $1\frac{1}{2}$ hours at 130° . The product was then poured into 12 c.c. of water containing a little sodium bisulphite. The yellow-orange precipitate was collected, washed with water and dried. A portion of the brown product was dissolved in alcohol and treated with a very small quantity of freshly prepared sodium amalgam. The solution immediately turned light brown and a bluish green flocky precipitate gradually appeared. No attempt was made to isolate the demethylated compound in a pure condition as we had not enough tambulin at our disposal.

The C—H estimations recorded in this paper have been carried out according to Pregl's method by Mr. N. Ghosh, M.Sc., to whom we offer our best thanks.

SUPPLEMENTARY RELATIONS BETWEEN THE PROTEINS OF PULSES AND THOSE OF MILK BY THE BALANCE- SHEET AND GROWTH METHODS.

BY K. P. BASU AND M. K. HALDAR.

Appreciable supplementary relation was found between the proteins of each of the pulses, *e. g.*, lentil (*Lens esculenta*), green gram (*Phaseolus mungo*), khesari (*Lathyrus sativa*), soya bean (*Glycine hispida*) and field pea (*Pisum sativa*) and those of milk, by the balance-sheet and growth methods, at 5 per cent level of protein intake, in the ratio, pulse proteins : milk proteins = 4 : 1 in the case of balance-sheet method and at 10 per cent level of intake, in the ratio, pulse proteins : milk proteins = 9 : 1, in the case of growth method. The condition of rats on mixed diets containing pulse and milk was better than the condition of those kept on pure pulse diets.

Pulses form a very important source of the proteins of the Indian dietaries. Investigations on the nutritive values of pulses carried out in this laboratory show that though they contain a high percentage of protein, the biological values are not very high (Basu *et al.*, *Indian J. Med. Res.*, 1936, **23**, 789, 881; 1937, **24**, 1001). Some of the pulses like lentil and *Lathyrus sativa* induce little or no growth in young rats. Previous investigations from this laboratory have shown that rice (Basu and Basak, *ibid.*, 1937, **24**, 1043) and fish proteins (Basu and De, *ibid.*, 1938, **26**, 177) have marked supplementary effect on pulse proteins. Sherman (*J. Biol. Chem.*, 1920, **41**, 97) and Sherman and Winters (*ibid.*, 1918, **36**, 301) worked on human objects and found that small amount of milk proteins has marked supplementary effect on cereal proteins. Mitchell (*J. Biol. Chem.*, 1924, **58**, 923) using rats, observed marked supplementary relations between corn and milk proteins in the proportion of 3:1 at a 10% level of intake. In the present paper the supplementary effect of the small additions of milk on the quality of pulse proteins has been investigated by the balance-sheet and growth methods. India is a poor country and the majority of the Indians cannot afford large quantities of milk and for that reason we worked with pulse and milk proteins in the proportion of 4:1 at a 5% level of protein intake in the balance-sheet experiment and at 9:1 ratio at a 10% level of protein intake in the growth method.

EXPERIMENTAL.

Five pulses, namely, lentil (*Lens esculenta*), green gram (*Phaseolus mungo*), *Lathyrus sativa*, soya bean (*Glycine hispida*), and field pea (*Pisum sativa*) were used in this investigation.

The milk powder was prepared by allowing the whole milk to dry in a large basin over a water-bath. The dried milk was kept in a vacuum desiccator for several days, then powdered in a mortar and kept in a vacuum desiccator and preserved in the refrigerator. Only small amounts of milk powder were prepared at a time and each sample was analysed before an experiment was made with it. On analysis it was found that the protein content of the milk powder varied from 22–24%, while the fat content varied from 26–34%. Before finding out the supplementary effect of milk on pulses, an experiment was carried out on rats with milk powder at 3% level of protein intake by the balance-sheet method and the biological value was found to be 93. Fixen and Jackson (1932), feeding rats with whole milk powder at 7% level, found 86 as the biological value. The lower value obtained by Fixen and Jackson is due to their working at a higher level of protein. That the biological value of a protein decreases with increase in protein concentration has been shown by Mitchell (*loc. cit.*) and by Basu and co-workers (*loc. cit.*) and also by Fixen. Experiments done by us with rations containing only 3% proteins from the milk powder failed to produce any growth in young rats. The composition of the unmixed diets is indicated in Table D₁ and that of the mixed diets in Table D₂.

TABLE D₁.*Composition of unmixed diets.*

Constituents of diets.	Milk powder.	Lentil.	Green gram.	<i>Lathyrus sativa</i> .	Soya bean.	Field pea.
Percentage of protein (approx)						
	3%.	10%.	10%.	10%.	10%.	10%
Milk powder	75 g.
Lentil	...	264 g.
Green gram	263 g.
<i>Lathyrus sativa</i>	186 g.
Soya bean	146 g.	...
Field pea	222 g.
Chopped sugar	54	54	54	54	54	54
Ghee	51	72	72	72	47	72
Cod liver oil	12	12	12	12	12	12
Salt mixture	24	24	24	24	24	24
CaCO ₃	6	6	6	6	6	6
Corn starch	378	168	169	246	311	210

TABLE D₃.*Composition of mixed diet.*

Constituents of diets.	→ % of Protein (approx)											
	N-free.	Lentil. milk = 4 1.	Lentil: milk = 9 1.	Green gram milk = 4 1	Green gram: milk = 9 1	<i>Lathyrus sativa</i> : milk = 4 1.	<i>Lathyrus sativa</i> . milk = 9 1.	Soya bean: milk = 4 1	Soya bean: milk = 9 1.	Field pea: milk = 4 1.	Field pea milk = 9 1	
Milk	...	5 25	10 27	5 25	10 27	5 26	10 26	5 25	10 27	5 26	10 27	
Lentil	...	107	244	
Green gram	103	232	
<i>Lathyrus sativa</i>	93	168	
Soya bean	58	131	
Field pea	111	199	
Chopped sugar	90 g	54	54	54	54	54	54	54	54	54	54	
Ghee (butter fat)	100	63	60	64	60	59	57	54	43	63	62	
Cod liver oil	20	12	12	12	12	12	12	12	12	12	12	
Salt mixture	50	24	24	24	24	24	24	25	24	24	24	
CaCO ₃	8	6	6	6	6	6	6	6	6	6	6	
Corn starch	735	309	173	312	185	326	253	367	303	304	216	

Experiments by the Balance-sheet Method.

Typical data obtained with 3% milk proteins and with mixtures of milk and lentil proteins in the ratio 1:4 at 5% level of protein intake are indicated in Tables I and II. Results obtained with other mixtures are summarised in Table III.

TABLE I.

Biological value of dried milk proteins at 3% level of intake.

(The figures of intake and output represent daily averages.)

Rat No.	Weight.		Intake		Nitrogen		Food N
	Initial.	Final.	Food.	Nitrogen.	Faecal.	Metabolic.	in faeces.
Experiment with nitrogen-free ration							
328	226 g.	224.7 g.	8.1 g.	...	16.57 mg.	*2.05 mg.	...
329	219	213.2	7.7	...	15.95	*2.07	.
330	144	143.0	6.0	...	7.88	*1.31	...
331	149	143.5	9.4	...	17.21	*1.83	...
332	119	115.4	8.8	...	15.90	*1.81	...
333	90	86.0	7.3	...	15.48	*2.12	...
Experiment with 3% milk protein.							
328	227	227.5	13.4	64.3 mg.	30.88	27.47	3.41 mg.
329	213	209.9	13.0	63.8	28.84	26.91	1.93
330	146	146.7	8.2	39.4	11.92	10.74	1.18
331	129	125.5	9.2	44.2	20.34	16.84	3.50
332	121	117.6	9.6	46.1	20.14	17.38	2.76
333	91	90.4	7.6	37.0	18.33	16.11	2.22
Rat No.	Absorbed food N.	Urinary N		Food N	Biological		Mean
		Total.	Endogenons.	in urine.	utilized.	value (B.V.)	B.V.
Experiment with nitrogen-free ration.							
328	44.60 mg.
329	28.59
330	37.69
331	38.06
332	30.00
333	..	.	32.51
Experiment with 3% milk protein.							
328	60.9 mg.	49.47 mg.	44.60	4.87	56.03	92	...
329	61.0	35.40	28.59	6.81	55.09	90	...
330	38.2	39.22	37.69	1.53	36.67	96	93
331	40.7	42.15	38.06	3.09	37.61	92	...
332	43.3	32.33	30.00	2.33	40.97	94	...
333	34.9	35.65	32.51	3.14	31.76	91	...

* per g. of food.

TABLE II.

Biological value of proteins of mixed diets containing lentil and milk protein in the ratio 4 : 1 at 5% level of intake.

(The figures of intake and output represent daily averages)

Rat No.	Weight		Food.	Intake N.	Nitrogen		Food N in faeces.	
	Initial	Final.			Faecal.	Metabolic.		
Experiment with nitrogen-free ration.								
286	314 g.	312.8 g.	13.1 g.	...	28.64 mg.	*2.19 mg.	...	
287	255	251.2	12.3	...	26.28	*2.20	...	
288	221	218.5	7.5	...	13.07	*1.74	...	
289	162	160.2	7.2	...	14.76	*2.05	...	
290	145	142.4	6.8	...	11.83	*1.74	...	
291	126	125.3	5.9	...	11.26	*1.91	...	
Experiment with mixed diet containing 5% protein (lentil : milk protein=4 : 1)								
286	316	316.0	13.6	109.3 mg.	40.99	29.73	11.26 mg.	
287	256	257.3	10.8	86.8	31.71	23.78	7.93	
288	219	219.7	11.7	92.0	30.41	20.39	10.02	
289	165	166.0	9.3	74.8	23.90	19.07	4.83	
290	145	145.8	9.0	73.5	22.71	15.66	7.05	
291	122	122.5	7.8	73.4	23.62	14.88	8.74	
Rat No.	Absorbed Food N.	Urinary N		Food N		Biological value (B. V.)	Mean B. V.	Calc. B. V.
		Total.	Endogenous.	In urine.	Utilized.			
Experiment with nitrogen-free ration.								
286	53.28 mg.
287	55.79
288	45.15
289	49.03
290	34.62
291	29.72
Experiment with mixed diet containing 5% protein (Lentil : milk protein=4 : 1)								
286	98.04 mg.	82.69 mg.	53.28	29.41 mg.	68.63 mg.	70		
287	78.87	77.00	55.79	22.21	56.66	72		
288	81.98	68.92	45.15	23.77	58.21	71	70	62
289	69.97	71.31	49.03	22.36	47.61	68		
290	66.45	55.25	34.62	20.63	45.82	69		
291	69.66	51.48	29.72	21.76	57.90	69		

* per g. of food.

TABLE III.

Biological value of mixed pulse and milk proteins (4:1) at 5% level.

Name of pulse	Observed value.	Calc. from individual values for pulse and milk.
<i>Lens esculenta</i> (lentil)	70	62
<i>Phaseolus mungo</i> (green gram)	74	70
<i>Lathyrus sativa</i> (khesari)	77	65
<i>Glycine hispida</i> (soya bean)	81	72
<i>Pisum sativa</i> (field pea)	69	63

Experiments by the Growth Method.

Typical growth data obtained with mixtures of *Lathyrus sativa* and milk proteins in the ratio 9 : 1 at 10% level of protein intake are indicated in Table IV. The results with all mixtures are summarised in Table V.

The values of growth per g. of protein intake with pulses alone and with mixtures of pulse and milk proteins (9 : 1) at 10% level are compared in Table VI.

• TABLE IV.

Growth experiments with mixed proteins of L. sativa and milk in the ratio 9:1 at 10% level of protein intake.

• F o u r w e e k s .							
Rat No	Sex.	Intake		Gain in wt.	* B. V.	Mean B.V.	
		Initial wt.	Food. Protein				
357	Male	55.0g	225.6g	22.56g.	1.2	1.1	
358	Female	47.5	182.6	18.26	1.1		
359	Male	52.5	214.3	21.43	1.1		
360	Female	49.5	211.6	21.16	1.0		
361	Male	48.0	266.4	26.64	1.1		
362	Male	47.5	216.1	21.61	1.2		

• E i g h t w e e k s .					
Rat No.	Intake		Gain in wt.	B.V.	Mean B.V.
	Food.	Protein.			
357	423.8g.	42.38g.	34.0g.	0.8	
358	398.4	39.84	25.5	0.6	
359	406.5	40.65	32.5	0.8	0.7
360	425.3	42.53	22.0	0.5	
361	428.3	42.83	30.0	0.7	
362	468.8	46.88	33.5	0.7	

$$* \text{ B. V. } = \frac{\text{gain in weight}}{\text{protein intake}}$$

TABLE V.

Biological value of proteins of mixed diets containing pulse and milk proteins in the ratio

pulse: milk 9:1 at 10% level of protein intake measured by the growth of young rats.

Source of protein.	Protein in the diet (per cent).	Number of rats.	Period of experi- ments. (weeks)	Increase in weight. Variation. Mean.	Total food intake. Variation. Mean.	Protein intake (mean).	B V
Mixed proteins of lentil (<i>Lens esculenta</i>) + milk.	10 (Lentil : milk = 9 : 1)	6	{ 4 8	22.0-27.5 g. 43.0-49.5	24.8 g. 46.8	173.1-195.2 g.* 375.3-418.7	18.61 g. 39.28
Lentil (<i>Lens esculenta</i>)	10	5	{ 4 8	4.0-18.0 5.0-18.5	11.2 11.1	58.6-137.0 115.2-256.0	9.96 18.23
Mixed proteins of green gram (<i>Phaseolus mungo</i>) + milk	10 (Green gram : milk = 9 : 1)	6	{ 4 8	27.0-36.5 58.5-68.0	33.5 63.0	179.3-216.1 408.7-457.7	19.72 44.27
Green gram (<i>Phaseolus mungo</i>)	10	5	{ 4 8	12.0-22.5 22.0-33.5	18.5 29.6	98.0-159.5 194.5-317.0	12.30 25.02
Mixed proteins of <i>Lathyrus sativa</i> + milk	10 (<i>Lathyrus sativa</i> : milk = 9 : 1)	6	{ 4 8	20.5-26.5 22.0-34.0	23.7 29.6	182.6-266.4 398.4-468.8	21.94 42.52
<i>Lathyrus sativa</i>	10	6	{ 4 8	0.0-4.6 0.0-10.4	1.5 4.3	62.2-85.4 103.2-168.3	7.24 12.73
Mixed proteins of soya bean (<i>Glycine hispida</i>) + milk	10 (Soya bean : milk = 9 : 1)	6	{ 4 8	32.5-42.5 59.5-74.0	37.8 68.2	201.5-238.2 457.7-571.4	21.04 52.03
Soya bean (<i>Glycine hispida</i>)	10	5	{ 4 8	29.7-41.2 57.0-70.8	33.5 57.5	157.4-285.5 362.6-522.4	19.64 44.16
Mixed proteins of field pea (<i>Pisum sativa</i>) + milk	10 (Field pea : milk = 9 : 1)	6	{ 4 8	17.0-21.5 24.5-32.0	19.3 27.1	102.5-146.9 204.1-244.8	12.40 22.28
Field pea (<i>Pisum sativa</i>)	10	6	{ 4 8	15.7-20.0 21.5-26.4	17.5 24.3	120.0-135.9 230.9-246.6	12.37 23.51

TABLE VI.

*Experiments by the growth method.**Growth per g. of protein intake with mixed diets containing pulse and milk proteins (9.1) at 10% level of protein intake.*

Name of pulse.	Growth per g. of protein.			
	Four weeks.		Eight weeks.	
	With pulse alone.	With pulse and milk.	With pulse alone.	With pulse and milk.
Lentil (<i>Lens esculenta</i>)	1.1	1.3	0.6	1.2
Green gram (<i>Phaseolus mungo</i>)	1.5	1.7	1.2	1.4
Khesari (<i>Lathyrus sativa</i>)	0.2	1.7	0.3	0.7
Soya bean (<i>Glycine hispida</i>)	1.7	1.8	1.3	1.3
Field pea (<i>Pisum sativa</i>)	1.4	1.6	1.0	1.2

DISCUSSION.

It will be evident from the above results that in each of the five pulses, when supplemented by milk at 5% level of protein intake and in the proportion, pulse proteins:milk proteins=4:1, the biological values, obtained by the balance-sheet experiment, are greater than the calculated values. Thus in the cases of *Lens esculenta*, *Phaseolus mungo*, *Lathyrus sativa*, soya bean, *Pisum sativa*, supplemented by milk, the corresponding observed and calculated biological values are 70 and 62, 74 and 70, 77 and 68, 81 and 72, and 69 and 63 respectively.

In growth experiments, the condition of rats on mixed diets was better than those on pure pulse diets; loss of hair was less apparent and the food intake and growth were greater with the mixed diets. With *Lathyrus sativa* and soya bean, the results are more interesting than others. The rats decreased in weight and showed loss of hair on a diet containing 10% proteins from *Lathyrus sativa* alone but when supplemented with milk this

pulse caused remarkably good growth, in rats and very little loss of hair could be observed. In the case of soya bean, addition of milk caused little or no improvement in the condition of rats. The ratio $\frac{\text{gain in weight (g.)}}{\text{protein intake (g.)}}$ in the case of rats kept on mixed diet, containing soya bean and milk, was almost the same for those on purely soya bean diet. In other cases this ratio is greater in mixed diets.

Recently Aykroyd and Krishnan (*Indian J. Med. Res.*, 1937, **24**, 1093 ; **25**, 367) have obtained enhanced growth in hostel boys and also in rats by the addition of small amounts of dried skimmed milk to the typical Madras diet and they attribute the beneficial results obtained to the calcium content and some heat-stable factor in vitamin B₂ complex of the milk. The results obtained in the present investigation show that the beneficial results are partly due to the supplementary effect of milk proteins on pulse proteins. The daily addition of two proteins of very high quality, viz., lactalbumin and caseinogen, in the form of 1 oz of dry skimmed milk is also bound to have a beneficial effect on growth and general well-being.

The necessity of incorporating at least small amounts of milk in the Indian dietaries is thus obvious.

CONCLUSION.

1. The biological value of proteins of whole milk at 3% level of protein intake is 93 by the balance-sheet method. With this ration, no growth in rats is obtained.

2. There is an appreciable supplementary relation by the balance sheet method, among the proteins of whole milk and proteins of each of the pulses, lentil, green gram, *lathyrus sativa*, soya bean and field pea at 5% level of intake, in the proportion pulse proteins : milk proteins = 4:1. The corresponding experimental and calculated biological values are 70 and 62, 74 and 70, 77 and 68, 81 and 72, and 69 and 63 respectively.

3. The growth per g. of protein intake is greater in the case of the mixed diets containing each of the above-mentioned pulses and milk, in the proportion, pulse proteins : milk proteins = 9:1, than the growth per g. of protein intake obtained from a pure pulse diet, in both the cases the level of protein intake being 10%. The only exception is soya bean where no supplementary effect can be found with milk, the growth per g. of protein

ingested being almost the same in the case of the mixed diet and pure soya bean diet.

4. Growth per g. of protein ingested for eight weeks of the mixed diet containing each of the five pulses, mentioned before, and milk, in the proportion of 9:1 at 10% protein level is as follows: lentil+milk, 1.2; green gram+milk, 1.4; *lathyrus sativa*+milk, 0.7; soya bean+milk, 1.3, and field pea+milk, 1.2. In the case of a pure pulse diet at the same concentration after eight weeks of feeding, the corresponding values are as follows: lentil, 0.6; green gram, 1.2; *lathyrus sativa*, 0.3; soya bean, 1.3, and field pea, 1.0.

5. The general condition of rats, kept on mixed diets mentioned above, is better than the condition those kept on the corresponding pure pulse diet. The loss of hair is less and the growth is greater in the former case.

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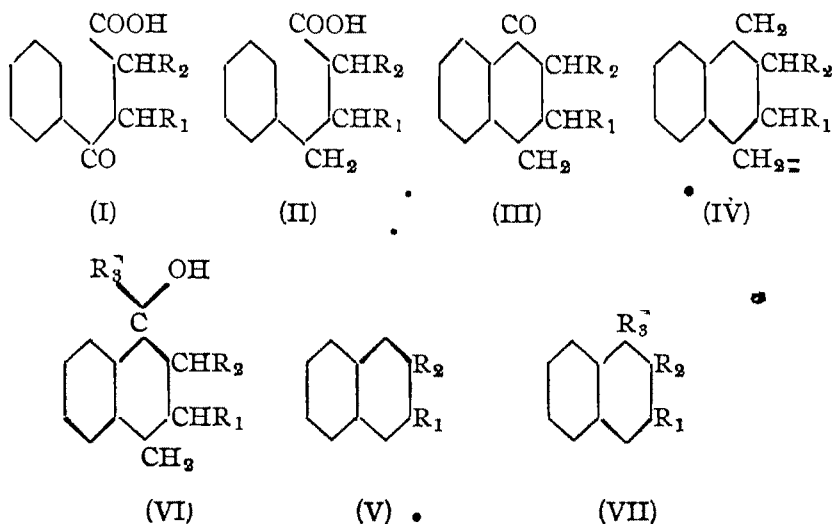
Received March 6, 1939.

STUDIES IN THE γ -KETONIC ACIDS, PART II.

BY P. C. MITTER AND LAKSHMI KANTA DE.

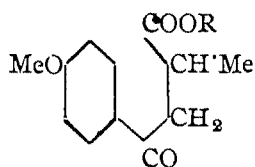
Methyl succinic anhydride has been condensed with phenol, anisole, resorcinol dimethyl ether and pyrogallol trimethyl ether giving rise to β -aroylpropionic acids, which have been reduced to the corresponding γ -arylbutyric acids. The latter have been cyclised to ketotetrahydronaphthalenes from which the corresponding naphthalenes have been obtained by dehydrogenation.

The condensation of methylsuccinic anhydride with aromatic hydrocarbons in the presence of aluminium chloride results in the production of a mixture of keto-acids (I, $R_1 = H$; $R_2 = Me$ and $R_1 = Me$; $R_2 = H$) which can be converted into substituted γ -arylbutyric acids (II, $R_1 = H$; $R_2 = Me$ and $R_1 = Me$; $R_2 = H$) by Clemmensen's method. On cyclisation the corresponding ketotetrahydronaphthalenes (III, $R_1 = H$; $R_2 = Me$ and $R_1 = Me$; $R_2 = H$) are produced. Further reduction by Clemmensen's method gives the tetrahydronaphthalene derivatives (IV, $R_1 = H$; $R_2 = Me$ and $R_1 = Me$; $R_2 = H$) whence by subsequent dehydrogenation by selenium the corresponding naphthalene hydrocarbons are obtained. Further alkyl groups can be introduced with the help of the Grignard reagent on the ketotetrahydronaphthalene derivatives. When the resulting carbinol (VI) is submitted to selenium dehydrogenation by simultaneous dehydration the naphthalene derivative (VII) is formed.

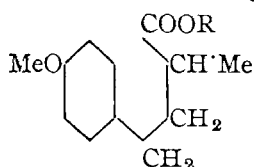


Mayer and Stamm (*Ber.*, 1923, **56**, 1424) condensed methyl succinic anhydride with benzene and obtained two isomeric products. Robertson and Waters (*J. Chem. Soc.*, 1933, 83) condensed veratrole with methylsuccinic anhydride in nitrobenzene and from the mixture of the isomeric products isolated 2:3-dimethoxy-6-methylnaphthalene by the scheme indicated above.

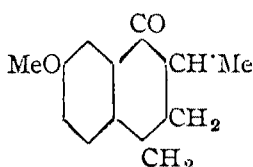
We have studied the condensation of phenol and various phenolic ethers with methylsuccinic anhydride with the object of synthesising various naphthalene derivatives with methyl groups in 6 or 7 positions. Anisole condenses with methylsuccinic anhydride in nitrobenzene solution at the ordinary temperature to give α -methyl- β -anisoylpropionic acid, m.p. 144°. Repeated fractional crystallisation of the condensation product from different solvents such as methyl alcohol, acetone, acetic acid and benzene, does not reveal the existence of an isomeric substance. The orientation of the methoxyl group has been proved by oxidising the product with moderately concentrated nitric acid in acetic acid solution to *p*-methoxybenzoic acid. A methyl alcoholic solution of the substance and salicylaldehyde at 0°, when saturated with hydrogen chloride, gives a bright red pyrylium derivative (Desai and Wali, *Proc. Indian Acad. Sci.*, 1937, **A**, **6**, 135). Therefore, the presence of a CO-CH₂ group is established. This reaction also fixes definitely the position of the methyl group.



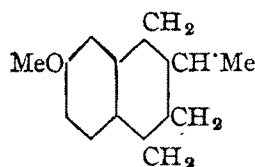
(VIII)



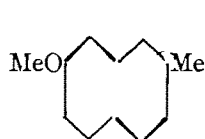
(XI)



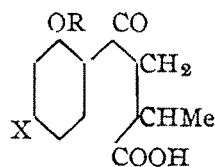
(XII)



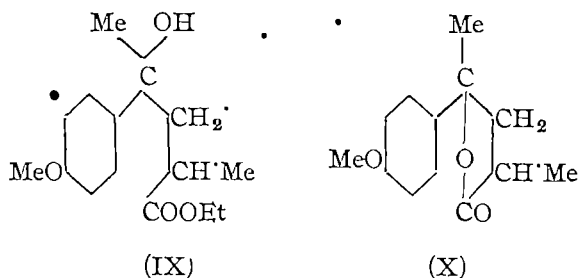
(XIII)



(XIV)



(XV)



The keto-acid (VIII, R=H) gives an ester (VIII, R=Et), b.p. 185-87°/5 mm. When the keto-ester is allowed to react with methyl magnesium iodide (1 mol.) two products have been obtained, the hydroxy-ester (IX) and a small amount of the γ -lactone (X). The lactone can not be reduced by zinc dust and 10% sodium hydroxide solution even when boiled for 20 hours.

The keto-acid on reduction by Clemmensen's method gives α -methyl- γ -(*p*-methoxy)-phenylbutyric acid (XI, R=H), b.p. 180-82°/5 mm. It gives an ester (XI, R=Et), b.p. 146-48°/4mm. Dehydration of the acid (XI, R=H) with phosphorus pentoxide gives 1-keto-1:2:3:4-tetrahydro-2-methyl-7-methoxynaphthalene (XII). The selenium dehydrogenation of the tetrahydronaphthalene (XIII) gives 2-methyl-7-methoxynaphthalene (XIV).

1-Keto-1:2:3:4-tetrahydro-7-methoxy-2-methylnaphthalene (XII) gives on condensation with methyl magnesium iodide, a mixture evidently of the carbinol and the related lactone which can not be separated.

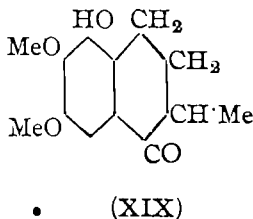
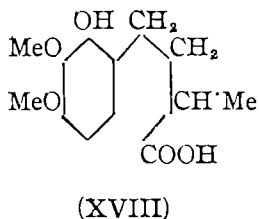
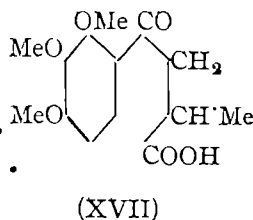
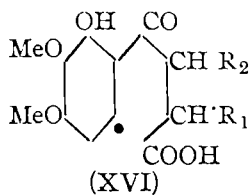
Phenol condenses with methylsuccinic anhydride in acetylene tetrachloride at 135-140° and gives α -methyl β -(*o*-hydroxy)-benzoylpropionic acid, m.p. 161° (XV, R=H; X=H). The product gives violet ferric reaction. The presence of a CO-CH₂ grouping in the keto-acid is proved by the formation of the pyrylium salt with salicylaldehyde and hydrogen chloride in methanol at 0°. The hydroxyketo-acid is methylated to α -methyl- β -(*o*-methoxy)-benzoylpropionic acid (XV, R=Me, X=H), m.p. 92-94°.

This substance is different from α -methyl- β -anisoylpropionic acid (VIII, R=H) whose constitution has been settled before. Further, the substance (m.p. 92-94°) has been proved to contain a CO-CH₂ grouping. Hence the condensation between phenol and methyl succinic anhydride has taken place in the *ortho* position with respect to the phenolic group. The keto-acid (XV, R=H; X=H) on Clemmensen's reduction gives α -methyl- γ -(*o*-hydroxy)-phenylbutyric acid (XV, R=H; X=H; CO replaced by CH₂), which does not cyclise with 85% sulphuric acid, but is sulphonated.

Resorcinol dimethyl ether condenses with methylsuccinic anhydride in nitrobenzene solution at the ordinary temperature to give a mixture of two substances. The mixture has been separated by repeated fractional crystallisation from methyl alcohol and acetone. The major portion of the condensation product crystallises in small needles, m.p. $130-31^{\circ}$. It gives reddish colouration with ferric chloride solution and a bright red pyrylium derivative is obtained with salicylaldehyde.

The second fraction crystallises in fine colourless needles, m.p. $141-42^{\circ}$, and gives faint pink colouration with ferric chloride showing that it has not been demethylated. The quantity is insufficient for an analysis. It is probably the second possible isomer. Following Perkin and Robinson (*J. Chem. Soc.*, 1908, 93, 506), we think the product (m.p. $130-31^{\circ}$) to be β -2 : 4-dimethoxybenzoyl- α -methylpropionic acid (XV, R=Me ; X=OMe).

Pyrogallol trimethyl ether in nitrobenzene solution gives a mixture of two substances. One fraction, which is the major portion of the condensation product, crystallises in light yellow needles (XVI, R_1 =Me ; R_2 =H), m.p. 155° , whilst the other component is a deep yellow needle-shaped crystalline solid, m.p. 175° (XVI, R_1 =H ; R_2 =Me). These compounds give violet colouration with ferric chloride solution which points to the fact that the condensation products have been demethylated. The presence of a CO-CH₂ grouping in the compound (m.p. 155°) has been proved by the formation of a pyrylium derivative. That one methoxyl group is hydrolysed during the reaction is confirmed by a Zeisel estimation. As the other isomer, m.p. 175° , does not form the pyrylium derivative it has been concluded that in this case the methyl group is in the β -position with respect to the carboxyl group.



By using methyl iodide and anhydrous potassium carbonate in dry acetone solution, the methyl ester of the trimethoxy derivative has been obtained which on hydrolysis gives α -methyl- γ -(2:3:4-trimethoxy)-benzoylbutyric acid (XVII). The keto-acid (XVI, $R_1 = \text{Me}$; $R_2 = \text{H}$) on reduction by Clemmensen's method yields α -methyl- γ -(2-hydroxy-3:4-dimethoxy)-phenylbutyric acid (XVIII). Dehydration of this acid with 85% sulphuric acid gives 1-keto-1:2:3:4-tetrahydro-2-methyl-6:7-dimethoxy-5-hydroxynaphthalene (XIX), m.p. 130-32°. Further reduction of this ring ketone by Clemmensen's method gives 1:2:3:4-tetrahydro-2-methyl-5-hydroxy-6:7-dimethoxynaphthalene (XIX, CO replaced by CH_2).

EXPERIMENTAL.

α -Methyl- β -anisoylpropionic Acid.—To a solution of powdered anhydrous aluminium chloride (180 g.) in nitrobenzene (500 c.c.), cooled in iced water, a mixture of methylsuccinic anhydride (70 g.) and anisole (70 g.) was added drop by drop. The flask was constantly shaken, and the mass became reddish brown and viscous towards the end. After standing overnight, the mixture was then poured into ice and dilute hydrochloric acid (1:1) with shaking. After the removal of nitrobenzene and unchanged anisole with steam, the residue solidified on cooling. It was collected and then digested with sodium carbonate solution. The filtrate on acidification in the cold, gave the keto-acid which crystallised from water in plates, m.p. 144°, yield 105 g. The acid was repeatedly crystallised fractionally from different solvents, such as ethyl alcohol, acetone, methyl alcohol, acetic acid, benzene, ethyl acetate but no evidence was obtained of a second isomer. (Found: C, 64.61; H, 6.21. $\text{C}_{12}\text{H}_{14}\text{O}_4$ requires C, 64.86, H, 6.30 per cent).

The *semicarbazone*, prepared in the usual way, crystallised from alcohol, m.p. 162° (decomp.). (Found: N, 15.23. $\text{C}_{13}\text{H}_{17}\text{O}_4\text{N}_3$ requires N, 15.05 per cent).

Oxidation of the Keto-acid (m.p. 144°) to 4-Methoxybenzoic Acid.—Moderately concentrated nitric acid (2 c.c.) was added to a solution of the keto-acid (0.8 g.) dissolved in acetic acid (3 c.c.). The mixture after heating for 1 hour on the steam-bath was diluted with water and the precipitated acid was crystallised from alcohol, m.p. and mixed m.p. with anisic acid, 183-84°.

Pyrylium Derivative of α -Methyl- β -anisoylpropionic Acid.—A mixture of the keto-acid (0.2 g.) and salicylaldehyde (0.2 g.) in methyl alcohol (15 c.c.) was saturated with dry hydrogen chloride at 0°. The mixture gradually

became deep red and the precipitate was filtered after 12 hours. It was washed with a little ethyl alcohol containing hydrogen chloride and dried in a vacuum desiccator. It is readily soluble in dilute alkali, and does not melt even at 300°.

Ethyl α-methyl-β-anisoylpropionate was obtained by esterifying the keto-acid in the usual way, b.p. 185-87°/5 mm. (Found: C, 67.43; H, 7.02. $C_{14}H_{18}O_4$ requires C, 67.20; H, 7.20 per cent).

α-Methyl-γ-(p-methoxy)-phenylbutyric Acid.—The keto-acid (50 g.) and zinc filings (100 g.) amalgamated with 5% mercuric chloride solution were refluxed with 200 c.c. of hydrochloric acid (d 1.19) for 10 hours. A further quantity of hydrochloric acid (100 c.c.) was introduced and the heating continued for 2 hours. After cooling the acid was extracted with ether, the extract washed with a little water and dried over anhydrous sodium sulphate. After the removal of ether, the residue was distilled at 180-82°/5 mm., yield 42 g. (Found: C, 69.47; H, 7.71. $C_{13}H_{16}O_3$ requires C, 69.23; H, 7.69 per cent).

Ethyl α-methyl-γ-(p-methoxy)-phenylbutyrate was prepared by esterifying the acid by Fischer's method, b. p. 146-48°/4.5 mm. (Found: C, 70.82; H, 8.65. $C_{14}H_{20}O_3$ requires C, 71.18; H, 8.47 per cent).

1-Keto-2-methyl-7-methoxy-1 : 2 : 3 : 4-tetrahydronaphthalene.—The reduced acid (26.5 g.) was added to phosphorus pentoxide (80 g.) and heated on the steam-bath for about 8 hours. The benzene solution was removed and the residual phosphorus pentoxide decomposed with ice. The contents of the flask were steam distilled and a small quantity of oil in the distillate was extracted with benzene. The combined benzene solution was washed with dilute sodium carbonate solution, water and then dried over calcium chloride. The residue after removal of benzene, was distilled in *vacuo* at 150-52°/5 mm. The distillate was a pale greenish liquid which became reddish after a few days, yield 20 g. (Found: C, 75.45; H, 7.40. $C_{12}H_{14}O_2$ requires C, 76.78; H, 7.36 per cent).

The ketone gave a *semicarbazone* under the usual conditions as brownish crystals from alcohol, m.p. 197°. (Found: N, 17.32. $C_{13}H_{17}O_2N_3$ requires N, 17.0 per cent).

1:2:3:4-Tetrahydro-2-methyl-7-methoxynaphthalene.—A mixture of the foregoing ketone (8 g.) amalgamated zinc (35 g.) and concentrated hydrochloric acid (85 c.c.) was refluxed for 15 hours. Concentrated hydrochloric acid was added at intervals. The product was extracted with ether; the ethereal extract was washed with dilute sodium carbonate solution and then with water. It was then dried over anhydrous sodium sulphate. The residue

after removal of ether was distilled at $114-115^\circ/5$ mm., yield 4.5 g. (Found : C, 81.97 ; H, 9.02. $C_{12}H_{16}O$ requires C, 81.81 ; H, 9.09 per cent).

2-Methyl-7-methoxynaphthalene.—Dehydrogenation was effected by heating a mixture of 2-methyl-7-methoxy-1:2:3:4-tetrahydronaphthalene (3.5 g.) and selenium (6 g.) for 25 hours at $310-320^\circ$. The product was extracted with ether, washed with cold 5% sodium hydroxide solution and then with water. The dried extract (sodium sulphate) gave a brownish residue after removal of ether, which was crystallised from alcohol in shining flakes, m.p. $89-90^\circ$, yield 2 g. (Found : C, 83.83 ; H, 6.90. $C_{12}H_{12}O$ requires C, 83.72 ; H, 6.97 per cent). The *picrate*, prepared in hot alcoholic solution, crystallised in orange needles, m.p. 119° . (Found : N, 10.23. $C_{18}H_{16}O_8N_3$ requires N, 10.47 per cent).

Action of Methyl Magnesium Iodide on 1-Keto-1:2:3:4-tetrahydro-7-methoxy-2-methylnaphthalene.—To the Grignard reagent prepared from magnesium ribbon (1.4 g.) and methyl iodide (4.5 c.c.) in dry ether, a solution of the ketone (10 g.) in dry ether was added dropwise with shaking and ice-cooling and then left overnight.

The solid magnesium compound was decomposed with ice-cold 10% dilute sulphuric acid and then extracted with ether, washed with water, then with sodium bisulphite and again with water. The dried ethereal extract (sodium sulphate) was freed from solvent and the residue, distilled at $134-38^\circ/5.5$ mm. The product decolourised potassium permanganate solution and was found by analysis to be a mixture of carbinol and dehydrated product, yield 8 g. (Found : C, 79.54 ; H, 8.62. $C_{13}H_{18}O_2$ requires C, 75.72 ; H, 8.73. $C_{13}H_{16}O$ requires C, 82.97 ; H, 8.51 per cent).

Condensation of Methyl Magnesium Iodide with Ethyl α -Methyl- β -anisoylpropionate.—To a solution of 25 g. of the ester in absolute ether (60 c. c.) was added the Grignard reagent prepared from magnesium powder (2.6 g.), methyl iodide (7.5 c. c.) and dry ether (20 c. c.) drop by drop with stirring. Reaction set in at once. After standing overnight the product was decomposed with dilute ice-cold hydrochloric acid. The ethereal extract after washing with dilute sodium bicarbonate solution, ice-cold 5% sodium hydroxide solution and water, was dried over sodium sulphate and the residue from ether distilled at $183-85^\circ/6$ mm., yield 15 g. (Found : C, 67.94 ; H, 8.34. $C_{15}H_{22}O_4$ requires C, 67.66 ; H, 8.27 per cent).

The sodium hydroxide washing on acidification gave an oily product which was extracted with ether, washed with water and dried over anhydrous sodium sulphate. On removal of ether, the product distilled at $175-77^\circ/5$ mm., yield 5 g. The lactone is insoluble in sodium bicarbonate

solution. (Found: C, 71.07; H, 7.45. $C_{13}H_{16}O_3$ requires C, 70.90; H, 7.27 per cent).

*α -Methyl- β -(*o*-hydroxy)-benzoylpropionic Acid.*—To a mixture of phenol (50 g.), methylsuccinic anhydride (50 g.) and acetylene tetrachloride (300 c. c.) powdered anhydrous aluminium chloride (150 g.) was gradually added with constant shaking, the mass becoming brown towards the end. The mixture was then heated in an oil-bath at 135–140° for 6 hours and kept overnight. The product was decomposed by the addition of crushed ice and hydrochloric acid (1 : 1) and then distilled in steam. The keto-acid solidified on cooling and was crystallised first from water and then from alcohol in colourless needles, m. p. 161°. No isomeric product was obtained on fractional crystallisation, yield 30 g. (Found: C, 63.61; H, 5.93. $C_{11}H_{12}O_4$ requires C, 63.46; H, 5.76 per cent). A poor yield was obtained when the reaction was carried out at the ordinary temperature. The methyl ether was obtained by methylating with dimethyl sulphate and caustic soda solution. It crystallised from ligroin, m. p. 92–94°. (Found: C, 64.7; H, 6.27. $C_{12}H_{14}O_4$ requires C, 64.86; H, 6.30 per cent).

*Pyrylium Derivative of α -Methyl- β -(*o*-hydroxy)-benzoylpropionic Acid.*—A mixture of the keto-acid (0.1 g.) and salicylaldehyde (0.1 g.) in methyl alcohol (8 c. c.) was saturated with dry hydrogen chloride at 0°. After a few minutes the mixture became deep red and was left overnight. It was then poured into water containing hydrochloric acid. The crimson red precipitate was filtered and washed with water and dried in a desiccator. It dissolved in alkali with bluish red colouration and did not melt up to 330°. *α -Methyl- γ -(*o*-hydroxy)-phenylbutyric acid* was obtained by Clemmensen reduction of the keto-acid, b. p. 170–73°/5.5 mm. (Found: C, 67.83; H, 7.35. $C_{11}H_{14}O_3$ requires C, 68.04; H, 7.21 per cent).

Condensation of Resorcinol Dimethyl Ether with Methylsuccinic Anhydride.—To a cold solution of powdered anhydrous aluminium chloride (30 g.) in nitrobenzene (80 c. c.) was added a mixture of methylsuccinic anhydride (10 g.) and resorcinol dimethyl ether (15 g.) dropwise. The flask was constantly shaken during the addition. The mass became reddish brown towards the end and was left overnight. The mixture was then poured into ice and dilute hydrochloric acid (1 : 1) with shaking and then subjected to steam distillation to remove nitrobenzene and unreacted resorcinol dimethyl ether. The residual semi-solid mass was extracted with ether, washed with a little water and the ether removed. On crystallisation from water it melted indefinitely between 115° and 124°, yield 11 g.

On repeated fractional crystallisation from methyl alcohol and acetic acid, two products were isolated viz., (i) pale brownish needles, m. p. 130–31°,

which gave reddish colouration with ferric chloride and (ii) colourless small needles, m. p. $142-143^\circ$, giving a very faint pink colouration with ferric chloride solution. [Found for the product (i): C, 61.66; H, 6.36. $C_{13}H_{16}O_5$ requires C, 61.90; H, 6.34 per cent].

When dry hydrogen chloride was passed to a methyl alcoholic solution of the substance (i) (0.1 g.) and salicylaldehyde (0.1 g.) the mixture gradually became deep red. After keeping overnight, it was poured into water when the pyrylium derivative was precipitated. It was filtered off, washed with water and dried in a desiccator. It did not melt up to 280° .

Condensation of Methylsuccinic Anhydride with Pyrogallol Trimethyl Ether.—A solution of aluminium chloride (210 g.) in nitrobenzene (600 c. c.) was cooled in ice-water and a mixture of methylsuccinic anhydride (75 g.) and pyrogallol trimethyl ether (105 g.) in nitrobenzene was slowly added to it. The mixture became brown and viscous and was left overnight. The aluminium chloride addition product was decomposed by ice-cold dilute hydrochloric acid (1 : 1) and then subjected to steam distillation to remove nitrobenzene and unreacted pyrogallol trimethyl ether. The residue solidified on cooling. It was filtered and extracted with hot sodium carbonate solution. The keto-acid was precipitated with hydrochloric acid in the cold and crystallised from water, m.p. $140-146^\circ$, yield 95 g.

After repeated fractional crystallisation from methyl alcohol, acetic acid and acetone two compounds were obtained, viz.

(i) Pale yellow needles, m.p. 155° , yield 18 g. (ii) Yellow needles, m.p. $175-176^\circ$, yield 6 g. Found for the compound (i): C, 58.33; H, 5.92; OMe, 23.46. $C_{13}H_{16}O_6$ requires C, 58.21; H, 5.97; OMe, 23.16 per cent. Found for the compound (ii): C, 58.46; H, 5.78. $C_{13}H_{16}O_6$ requires C, 58.21; H, 5.97 per cent. The substance (i) gave a pyrylium derivative, whilst (ii) did not.

The *semicarbazone* of the keto-acid (i) crystallised from alcohol, m.p. $208-9^\circ$. (Found: N, 12.65. $C_{14}H_{18}O_6N_2$ requires N, 12.92 per cent).

The acid was methylated in acetone solution with methyl iodide in presence of dry potassium carbonate. It crystallised from petroleum ether, m.p. $89-90^\circ$. (Found: C, 59.73; H, 6.57. $C_{14}H_{18}O_6$ requires C, 59.57; H, 6.38 per cent).

On Clemmensen's reduction, the keto-acid gave α -methyl- γ -(2-hydroxy-3:4-dimethoxy)-phenylbutyric acid as very fine needles from petroleum ether, m.p. $83-85^\circ$. (Found: C, 61.62; H, 7.06. $C_{13}H_{18}O_6$ requires C, 61.41; H, 7.08 per cent).

1-Keto-1:2:3:4-tetrahydro-2-methyl-5-hydroxy-6:7-dimethoxynaphthalene.—A mixture (35 c. c.) of 85 g. of pure sulphuric acid and 15 c. c. of

water was added to the reduced acid (8 g.) and the mixture heated on the water-bath for $1\frac{1}{2}$ hours. After cooling the mixture was poured into crushed ice and water and the product was extracted with ether. The ethereal extract was washed with water, then with sodium bicarbonate solution and finally with water, and then dried (sodium sulphate). After removal of ether the residue was crystallised from dilute methyl alcohol in needles, m.p. $130-32^{\circ}$, yield 6 g. (Found: C, 66.01; H, 6.91. $C_{13}H_{16}O_4$ requires C, 66.10; H, 6.78 per cent).

The *semicarbazone* crystallised from alcohol, m.p. $228-29^{\circ}$. (Found: N, 14.74. $C_{14}H_{19}O_4N_3$ requires N, 14.33 per cent).

1:2:3:4-Tetrahydro-2-methyl-5-hydroxy-6:7-dimethoxynaphthalene.—The ring ketone (5.5 g.) was reduced with amalgamated zinc (30 g.) and concentrated hydrochloric acid (80 c.c.), by heating for 15 hours, hydrochloric acid (5 c.c.) being added every hour. The product was extracted with ether, the ethereal extract was washed with a dilute solution of sodium bicarbonate and with water, and then dried over anhydrous sodium sulphate. The residue from ether distilled at $152-54^{\circ}/6$ mm. The distillate was a colourless liquid which solidified when kept in a vacuum desiccator, yield 3 g. (Found: C, 70.41; H, 8.35. $C_{13}H_{18}O_3$ requires C, 70.27; H, 8.11 per cent).

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Received March 14, 1939.

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BIOLOGICAL VALUE OF THE PROTEINS OF *CICER*
ARIETINUM (BENGAL GRAM) AND *CAJANUS*
INDICUS (ARHAR) BY THE BALANCE-
SHEET AND GROWTH METHODS.

BY K. P. BASU AND M. K. HALDAR

The biological value of proteins of *Cicer arietinum* (Bengal gram) and *Cajanus indicus* (Arhar) was determined by the balance-sheet method and by the growth of young rats. In the balance-sheet experiments, *Cicer arietinum* has a higher biological value than the value of *Cajanus indicus*. In the growth method, the growth of rat per gramme of protein intake produced by *Cicer arietinum* at 15 per cent concentration of protein is less than the corresponding value for *Cajanus indicus*, while at lower concentrations of proteins, *Cicer arietinum* produces more growth.

Investigations on the biological values and chemical analysis of proteins of different varieties of rice, pulse and fish have been pursued for a number of years in this laboratory (Basu *et al.*, *Indian J. Med. Res.*, 1936, 28, 789, 811; 1937, 24, 1001, 1043, 1067; 1938, 26, 177, 191). These investigations have shown that *Aman* rice proteins are much superior to *Aus* rice proteins in promoting growth, that Ruhee (*Labeo rohita*) proteins are of a better quality than Hilsa (*Clupea ilisa*) proteins and that contrary to the popular belief, proteins of green gram have higher biological value than those of lentil and produce much more rapid growth in young rats than the latter. It has been found that *Lathyrus sativa*, while not of much value in promoting growth, induces no lathyrism in rats. The biological value of different pulses has been found to be different and in some cases pulses, which appear to be equally efficient in maintaining nitrogenous equilibrium, behaved quite differently in promoting growth. The present investigation deals with the nutritive value of the two common pulses *Cicer arietinum* (Bengal gram) and *Cajanus indicus* (Arhar). The experiments were carried out with rats and the technique employed in both the balance-sheet and growth methods was the same as followed in previous investigations from this laboratory.

The composition of the pulses used as sources of protein is given in Table I and that of the diets in Table II.

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TABLE I.

Analysis of pulses used as sources of protein.

Pulse.	Moisture.	Total N.	Protein N $\times 6.25$	Ether extractive.	Ash.	Crude fibre.	Carbohydrate by diff.
<i>Cicer arietinum</i>	15.11%	3.82%	23.87%	5.75%	2.83%	3.24%	49.20%
<i>Cajanus indicus</i>	10.35	3.87	24.19	1.89	3.35	1.01	59.21

TABLE II.

Composition of diets.

Constituents of diets.	Nitrogen-free.	<i>Cicer arietinum.</i>			<i>Cajanus indicus.</i>		
		Percentage of protein (approx.) in diet					
	...	5%.	10%.	15%.	5%.	10%.	15%.
<i>Cicer arietinum</i>	0 g.	126 g.	252 g.	378 g.
<i>Cajanus indicus</i>	0	129 g.	259 g.	388 g.
Chopped sugar	90	54	54	54	54	54	54
Ghee (butter fat)	100	65	58	50	72	72	72
Cod liver oil	20	12	12	12	12	12	12
Salt mixture	50	24	24	24	24	24	24
Calcium carbonate	8	6	6	6	6	6	6
Corn starch	735	313	194	76	303	173	44

EXPERIMENTAL.

The Balance-sheet Method.

Typical data regarding the metabolism experiments and calculations of the biological values for 10% protein level of the two pulses *Cicer arietinum* and *Cajanus indicus* proteins in the diet are indicated in Tables III and IV. The results of experiments at different concentrations of proteins in the diet are summarised in Table V.

TABLE III.

Balance-sheet experiments with proteins of Cicer arietinum.

(Figures of intake and excretion represent daily averages.)

Rat No	Weight		Intake		Nitrogen		Food nitrogen	
	Initial.	Final.	Food.	Nitrogen.	Faecal.	Metabolic.	In faeces.	Absorbed
Experiments with nitrogen-free diet.								
575	197 g.	195.5 g.	12.0 g.	...	13.54 mg.	*1.13 mg.
576	212	211.4	12.0	...	16.54	*1.38
577	215	212.8	10.2	...	12.95	*1.27
578	198	196.6	8.8	...	14.15	*1.61
579	217	216.1	11.9	...	17.52	*1.47
580	194	188.4	11.2	...	22.02	*1.97

Experiments with 10% *Cicer arietinum* protein.

575	195 g.	196.4 g.	13.5 g.	227.61 mg	47.73 g.	15.26 g.	32.47 mg.	195.14 mg
576	215	215.8	13.5	227.61	56.31	18.63	37.68	189.93
577	215	216.2	14.1	237.61	45.38*	17.91	27.47	210.14
578	199	201.2	13.0	217.42	48.04	20.93	27.11	190.31
579	219	219.7	13.2	218.18	60.53	19.40	41.13	187.05
580	195	197.3	12.2	203.76	61.41	24.03	37.38	166.38

Rat No.	Digestibility.	Mean digestibility.	Urinary N		Food nitrogen		Biological value (B.V.)	Mean B.V.
			Total.	Endogenous.	in urine.	Utilised.		

Experiments with nitrogen free-diet.

575	32.58 mg.
576	32.46
577	37.70
578	31.21
579	47.73
580	44.97

Experiments with 10% *Cicer arietinum* protein.

575	86		123.83 mg.	32.58 mg.	91.25 mg.	103.89 mg.	53	
576	83		118.01	32.46	85.55	104.38	54	
577	88	85	142.17	37.70	104.47	105.67	50	52
578	88		127.30	31.21	96.09	94.22	50	
579	82		133.69	47.73	85.96	101.09	54	
580	82		125.40	44.97	80.43	85.95	52	

* per g. of food intake.

TABLE IV.

Balance-sheet experiments with proteins of Cajanus indicus.

(Figures of intake and excretion represent daily averages).

Rat No.	Weight		Intake		Nitrogen		Food	
	Initial.	Final.	Food.	Nitrogen.	Faecal	Metabolic.	In faeces.	Absorbed.

Experiments with nitrogen-free ration.

581	218 g.	216.4 g	9.4 g.	.	17.21 mg	*1.84 mg
582	322	319.2	13.2	...	18.67	*1.42
583	304	301.6	13.0	..	20.39	*1.56
584	286	284.2	12.5	...	22.43	*1.79
585	254	252.4	10.8	...	16.18	*1.50
586	235	232.1	11.4	.	21.55	*1.89

Experiments with 10% *Cajanus indicus* protein.

581	217 g.	218.5 g.	9.5 g.	153.85 mg.	35.79 mg.	17.48 mg.	18.31 mg.	140.54 mg.
582	324	324.6	13.4	210.37	33.68	19.03	14.65	195.72
583	309	309.9	14.5	229.66	40.93	22.62	18.31	211.35
584	291	289.3	14.4	228.43	41.69	25.78	15.91	212.52
585	261	262.8	11.0	180.08	32.61	16.50	16.11	163.97
586	239	238.6	12.7	205.16	40.48	24.00	16.48	188.68

Experiments with nitrogen-free ration.

Rat No.	Digestibility.	Mean digestibility	Urinary N		Food nitrogen in urine.	utilized	Biological value (B. V.)	Mean B. V.
			Total.	Endogenous.				
581	38.14 mg.
582	51.78
583	54.25
584	42.72
585	44.69
586	49.36

Experiments with 10% *Cajanus indicus* protein.

581	88		109.67 mg	38.14 mg	71.53 mg.	69.01 mg.	49	
582	93		161.25	51.78	109.47	86.25	44	
583	92	92	168.18	54.25	113.93	97.42	46	46
584	93		146.66	42.72	103.94	108.58	51	
585	91		137.52	44.69	92.83	71.14	43	
586	92		154.45	49.36	105.39	83.09	44	

* per g. of food intake.

TABLE V.

Biological value and Digestibility of proteins of Cicer arietinum and Cajanus indicus.

Material.	Rat No.	Body wt	Percentage of proteins in the diet.					
			5%.		10%.		15%.	
			B.V.	D.	B.V.	D.	B.V.	D.
C. Arietinum.	575	197 g.	59	80	53	86	41	90
	576	212	57	87	54	83	46	89
	577	215	59	83	50	88	43	86
	578	198	61	84	50	88	49	84
	579	217	58	87	54	82	46	90
	580	194	64	86	52	82	48	86
	Average		60	85	52	85	46	88
C. Indicus.	581	218	53	75	49	88	32	82
	582	322	57	76	44	93	37	84
	583	304	52	73	46	92	36	84
	584	286	56	78	51	93	34	82
	585	254	59	74	43	91	41	84
	586	235	54	77	44	92	34	82
	Average		55	76	46	92	36	83

The mean biological value (B.V.), digestibility (D) and protein values of proteins of different pulses at 10% level of proteins determined in this laboratory are summarised in Table VI for the sake of comparison.

TABLE VI.

Pulse.	Protein content	Mean biological value.	Mean digestibility.	Mean protein value.
	P.	B.V.	D.	$= P \times \frac{B.V.}{100} \times \frac{D}{100}$
<i>Lens esculenta</i> (Lentil) ...	22.60 %.	32	90	6.5
<i>Phaseolus mungo</i> (Green gram)	23.26	52	86	10.4
<i>Glycine hispida</i> (Soya bean) ...	41.20	58	85	20.2
<i>Lathyrus sativa</i> (Khesari) ...	32.20	50	90	14.4
<i>Pisum sativa</i> (Field pea) ...	27.10	48	91	11.7
<i>Cicer arietinum</i> (Bengal gram)	23.87	52	85	10.6
<i>Cajanus indicus</i> (Arhar) ...	24.19	46	92	10.2

The Growth Method.

The quality of the two pulses has also been determined by the growth produced in young growing rats per g. of protein intake over a period of four and eight weeks. The levels of protein intake and the composition of diets were the same as in the balance-sheet experiments. The same method was adopted as was done in previous investigations in this laboratory. The growths obtained and the biological values (B.D.)

$\left[= \frac{\text{gain in weight (g.)}}{\text{protein intake (g.)}} \right]$ at 10 per cent protein level are indicated in Table

VII and the results at different protein concentrations are summarised in Table VIII.

TABLE VII.

Growth experiments with proteins of Cicer arietinum and Cajanus indicus at 10% protein level.

Rat No.	Sex.	Initial weight.	Four weeks.				Eight weeks.				Mean B. V.
			Food.	Intake Protein.	Gain in wt.	*B. V.	Food.	Intake Protein.	Gain in wt.	*B. V.	
10% <i>Cicer arietinum</i> protein in diet.											
520	Female	50 g.	183.4 g.	18.34 g.	23.5 g.	1.3	382.2 g.	38.22 g.	45.0 g.	1.2	
521	Male	50	205.2.	20.52	36.0	1.7	508.4	50.84	72.0	1.4	1.3
522	Male	50	196.0	19.60	31.0	1.6	434.5	43.45	65.5	1.5	
523	Female	50	180.3	18.03	25.5	1.4	452.8	45.28	51.0	1.1	
524	Female	45.5	165.8	16.58	23.0	1.4	395.6	39.56	52.5	1.3	
525	Female	48.5	148.5	14.85	23.5	1.6	413.7	41.37	48.0	1.2	
10% <i>Cajanus indicus</i> protein.											
555	Male	47.5	192.4	19.24	27.0	1.4	403.8	40.38	44.5	1.1	
556	Female	46.5	168.7	16.87	21.5	1.3	346.5	34.65	28.0	0.8	
557	Female	42.0	174.5	17.45	24.5*	1.4	384.9	38.49	38.0	1.0	1.0
558	Male	43.5	183.5	18.35	27.5	1.5	414.6	41.46	49.5	1.2	
559	Male	41.0	191.3	19.13	26.0	1.4	422.3	42.23	42.0	1.0	
660	Male	47.5	178.7	17.87	26.5	1.5	365.7	36.57	40.5	1.1	

* B. V. = Gain in wt./protein intake.

TABLE VIII.

Mean biological values of proteins of C. arietinum and C. indicus by the growth method.

Protein in the diet.	Number of rats.	Period of experiments.	Increase in weight • Variation.	Mean.	Total food intake (dry). Variation.	Protein intake (mean).	B V.
<i>Cicer arietinum</i> .							
%	6	4 (weeks).	10.5-15.5 g.	12.8 g	142.8-162.9 g.	153.7 g.	1.7
		8	8.5-15.5	11.3.	238.4-296.4	275.0	0.8
	6	4	23.0-36.0	27.1	148.5-205.2	179.9	1.5
		8	45.0-72.0	55.7	382.2-508.4	431.2	1.3
15	6	4	25.5-44.5	34.3	163.8-183.5	173.5	1.3
		8	41.0-80.0	60.7	327.9-403.0	358.3	1.1
<i>Cajanus indicus</i> .							
5	6	4	(-4.0)-1.5	- 0.4	116.0-140.7	131.7	...
		8	(-4.5)-0.5	- 2.4	245.1-280.2	266.9	...
10	6	4	21.5-27.5	25.5	168.7-192.4	181.5	1.4
		8	28.0-49.5	40.4	346.5-422.2	389.6	1.0
15	6	4	39.5-52.0	45.3	166.8-192.5	179.6	1.7
		8	63.5-91.0	75.2	341.3-382.5	363.1	1.4

For the sake of comparison the biological values of the proteins of the different pulses by the growth method worked out in this laboratory are given in Table IX.

TABLE IX.

Pulse.	Growth per g. of protein intake after	
	four weeks.	eight weeks.
<i>Lens esculenta</i> (Lentil)	1.1	0.9
<i>Phaseolus mungo</i> (green Gram)	1.4	1.2
<i>Lathyrus sativa</i> (Khesari)	0.6	No growth
<i>Glycine hispida</i> (Soya bean)	1.9	1.5
<i>Pisum sativum</i> (Field pea)	1.5	0.9
<i>Cicer arietinum</i> (Bengal gram)	1.5	1.3
<i>Cajanus indicus</i> (Arhar)	1.4	1.0

DISCUSSION.

Results obtained indicate that the proteins of *Cicer arietinum* are superior to those of *Cajanus indicus* both for the maintenance of nitrogen balance as well as for promoting growth in young rats, though the latter contains a higher percentage of protein than the former. With respect to biological value, digestibility and protein value *Cicer arietinum* is as efficient as *Phaseolus mungo*. The biological value of *Cajanus indicus* is rather low, being higher than that of *Lens esculenta* only but the digestibility of the proteins of *Lens esculenta* is higher than those of *Cajanus indicus*.

In the growth experiments the proteins of *Cicer arietinum* caused appreciable growth at 5% level of intake, while the proteins of *Cajanus indicus* at the same concentration produced little or no growth. It is evident that the proteins of *Cajanus indicus* are deficient in one or more of the essential amino-acids. At 10% level of intake also, the proteins of *Cicer arietinum* are superior to those of *Cajanus indicus*. It is interesting to note that at 15% level of intake, the proteins of *Cicer arietinum* are inferior to those of *Cajanus indicus* in promoting growth, though the food intake was almost the same in both the cases. The reason is not quite clear, but it may be possible that though *Cicer arietinum* proteins are superior to those of *Cajanus indicus*, probably the former may contain in minute amounts some factor which inhibits growth. The action of this growth-inhibitory factor is not

so marked at lower concentration of the pulse in the diet but may become appreciable at higher concentrations. Growth per g. of protein intake with *Cicer arietinum* increases from 0.8 at 5% protein level to 1.3 at 10% level because 5% protein in the diet are quite an insufficient amount of protein and the effect of the toxin is more than counterbalanced by the increase in protein intake. As the percentage of protein is, however, increased to 15, growth per g. of protein intake decreases to 1.1 very probably due to the effect of the growth-inhibitory factor. With *Cajanus indicus* growth per g. of protein-intake continually increases from 0 to 1.4 as the percentage of protein in the diet increases from 5 to 15.

CONCLUSION

The biological values of proteins of *Cicer arietinum* by the balance-sheet method at 5, 10 and 15% levels of feeding are 60, 52 and 46 respectively. The corresponding values for *Cajanus indicus* are 55, 46 and 36. *Cicer arietinum* has thus a higher biological value than *Cajanus indicus* at all levels of intake and with a particular pulse the biological value decreases with increase in concentration of protein in the diet.

The mean digestibilities of the protein of *Cicer arietinum* at 5, 10 and 15% levels of intake are 85, 85 and 88 respectively and the corresponding values for *Cajanus indicus* are 76, 92 and 83. The protein values of *Cicer arietinum* and *Cajanus indicus* at 10% level of intake are practically identical, the values being 10.6 and 10.2 respectively.

Growth per g. of protein ingested at 15% protein concentration in the diet is for *Cicer arietinum* 1.1 and for *Cajanus indicus*, 1.4. With 10% protein the corresponding values for *Cicer arietinum* and *Cajanus indicus* are 1.3 and 1.0 respectively. With 5% concentration of protein growth per g. of protein ingested is 0.8 in the case of *Cicer arietinum* while *Cajanus indicus* does not produce any growth at that concentration. *Cicer arietinum* may contain traces of a growth-inhibitory factor which makes its presence felt at higher intakes of proteins.

ON THE PREPARATION OF 7-METHYL-3-HYDROXYTHIONAPHTHENE AND ITS CONDENSATION WITH ISATIN.

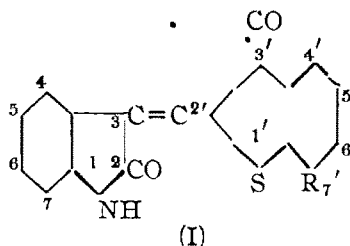
BY SISIR KUMAR GUHA.

7-Methyl-3-hydroxythionaphthene has been prepared and condensed with isatin. 3-Indole-2'-(7'-methyl)-thionaphtheneindigo, thus obtained, has been compared with its isomeric 4', 5', and 6'-methyl derivatives, prepared before by the present author and also the parent compound, Thioindigo Scarlet R.

It appears that 7-methyl-3-hydroxythionaphthene has not yet been described in literature although the possibility of obtaining its oxidation product, 7:7'-dimethylthioindigo, starting from *o*-toluidine, has been mentioned in D. R. P. 241910 which does not give any particulars regarding its preparation and properties (*cf.* Thorpe and Ingold, "Vat Colours," 1923, p. 129).

The present author required 7-methyl-3-hydroxythionaphthene, in order to complete the study of indole-methylthionaphtheneindigos, methylthionaphthene-acenaphthyleneindigos, bismethylthionaphtheneethyleneindigos, and benzylidenemethylthionaphthenes (Guha and Basu-Mallick, *J. Indian Chem. Soc.*, 1934, **11**, 395; Guha, *ibid.*, 1937, **14**, 240; 1938, **15**, 501; 1933, **10**, 679; 1936, **13**, 94; 1938, **15**, 20; 1935, **12**, 659; 1937, **14**, 709; 1938, **15**, 359; *cf.* Auwers and Arndt, *Ber.*, 1909, **42**, 551). It has been obtained from *o*-methylphenylthioglycollic acid by the action of phosphorus pentoxide by following the method of preparation of 5-chloro-3-hydroxythionaphthene (D. R. P. 224567; Friedlander, **10**, 474; *cf.* Auwers and Thies, *Ber.*, 1920, **53**, 2285). The isolation of this substance has made the path open and easy for the synthesis of a large number of valuable thioindigoid vat dyes in the isatin, acenaphthenequinone, phenanthrenequinone, conjugated indigo analogues and thioindogenide series just in the same way as already described by the present author in the case of 4-, 5-, and 6-methyl-3-hydroxythionaphthene (Guha, *loc. cit.*; Guha and Basu-Mallick; *loc. cit.*) and also in other series by several other workers (F. P. 693903; *Chem. Zentr.*, 1931, **I**, 102, 2944; U. S. P. 1850758, *Chem. Zentr.*, 1932, **103**, **II**, 1374; *Ber.*, 1914, **47**, 955; 1923, **56**, 1308; 1925, **58**, 824; *Annalen*, 1925, **443**, 211).

When isatin is condensed with 7-methyl-3-hydroxythionaphthene, 3-indole-2'-(7'-methyl)-thionaphtheneindigo (I, R=Me) is obtained.



Its colour and dyeing properties have been studied. The absorption spectra has been taken in xylene solution. The microphotogram (Fig. 1) has been drawn and the absorption maxima calculated from the graph. It is found (*cf.* Table I) that this 7'-methyl dye is lighter than the parent compound, commercially known as Thioindigo Scarlet R (I, R=H), *i.e.*, when the Me group is introduced into the *ortho* position to the indigo auxochrome S, the colour of the mother substance is lightened. This effect of the influence of the Me group in the 7'-position, which has been studied for the first time now, and also the effect of the Me group in the 4'-, 5'- and 6'- positions (Guha, *loc. cit.*) has enabled the author to generalise that when the Me group is introduced in the 4'-, 6'- and 7'-positions, the colour of the parent compound is lightened, and when the same group occupies the 5'-position the colour of the mother substances is deepened; *i.e.*, lightening and deepening of colour of the mother substance take place alternately as the Me group is shifted from the 4'- to the 6'-position and it is further lightened when the same group is again shifted from the 6'- to the 7'-position of the thionaphthene nucleus of the molecule of 3-indole-2'-thionaphtheneindigo (I, R=H).

The absorption maxima of 3-indole 2'-thionaphtheneindigo and its four theoretically possible isomeric methyl derivatives, having the Me group in the thionaphthene nucleus of the molecule, are given in Table I for comparison.

TABLE I.

T=Thionaphtheneindigo.

Compounds		Absorption maxima
3-Indole-2'-T	...	4892 Å
3-Indole-2'-(4'-Me)-T	.	4885
3-Indole-2'-(5'-Me)-T	...	4986
3-Indole-2'-(6'-Me)-T	...	4880
3-Indole-2'-(7'-Me)-T	..	4848

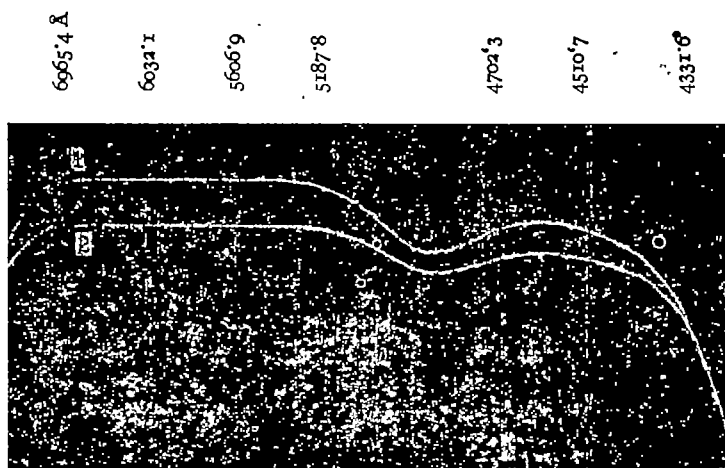
A detailed study of this class of dyes in various series has been undertaken.

EXPERIMENTAL.

7-Methyl-3-hydroxythionaphthene.—*o*-Methylphenylthioglycollic acid (10 g.) was heated in a hard glass test tube in an oil-bath at 150°. To the molten substance, phosphorus pentoxide (15 g.) was added, and the mixture stirred well for 1 hour at 150-160°, and allowed to cool. The hard mass was digested well with sodium hydroxide (2*N* approx) and the resulting solution acidified with hydrochloric acid, 7-methyl-3-hydroxythionaphthene being removed by distilling in steam. It was collected as a colourless heavy oily liquid which turned pale yellow. By allowing it to stand in ice, the oily substance solidified which was at first dried in a vacuum desiccator and finally on a porous plate, when it became colourless, m. p. 68-69°. It is a rectangular crystalline substance, soluble in pyridine, nitrobenzene, aniline, acetic acid, alcohol and ether. (Found: S, 19.41. C_9H_8OS requires S, 19.51 per cent).

3-Indole-2'-(7'-methyl)-thionaphtheneindigo.—The dark red solution, produced by boiling isatin (0.574 g.) and 7-methyl-3-hydroxythionaphthene (0.641 g.) in 43 c.c. of glacial acetic acid and 4 c.c. of concentrated hydrochloric acid for 15-20 minutes, was allowed to stand overnight. The deep red crystalline precipitate was collected and crystallised from xylene in bundles of long silky bright scarlet needles or from alcohol in dark red needles, m.p. 314°. It is soluble in acetic acid, alcohol and difficultly soluble

FIG. 1.



Curves 1 and 2 refer to 3-indole-2'-(7'-methyl)-thionaphtheneindigo; corresponding exposure being (1) 15' and (2) 20' respectively.

in xylene. Cold concentrated sulphuric acid dissolves it with a dark brown colour but the solution in hot concentrated sulphuric acid is green. It dyes wool in dark red shade from an acid bath and cotton in light red colour from a light yellow alkaline hydrosulphite vat. (Found: S, 11.04. $C_{17}H_{11}O_2NS$ requires S, 10.92 per cent).

My thanks are due to Prof. K. Proshad for taking interest in this work and to Messrs D. K. Bhattacharya and B N. Ghosh for helping me with spectrograph and microphotometer.

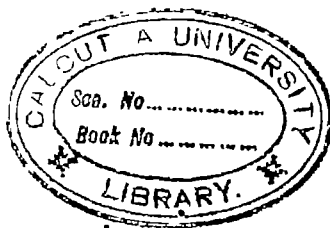
SCIENCE COLLEGE,
PATNA.

Received March 23, 1939.

ERRATA.

Page	Line	Read	for
67	8	being	is
	Formula I	$\text{HO}_2\text{C}\cdot\text{CH}=\overset{\text{CHMe}_2}{\underset{ }{\text{C}}}-\text{CH}_2\cdot\text{CO}_2\text{H}$	
	„ (II)	$\text{H}_2\text{C}\begin{cases} \text{CHMe}_2 \\ \\ \text{C}\cdot\text{CH}_2\cdot\text{CO}_2\text{H} \\ \\ \text{CH}\cdot\text{CO}_2\text{H} \end{cases}$	
69	7 ¹	13 g.	1'3 g.
100	Caption	Part III.	Part II.
107	5 ³	1935	1835
108	12	(VI, R=c, h.)	(VI, R=e h.)
126	8	carbonyl	carboxyl
	1*	thioindogenide	thioindigenide
128	11	dyeing	dying
129	11	violet	voilet

* From bottom.



THE ELECTRICAL CONDUCTIVITY OF SOLUTIONS CONTAINING ZINC HYDROXIDE AND SODIUM HYDROXIDE.

BY S. M. MEHTA AND M. B. KABADI.

The measurement of electrical conductivity of solutions containing ZnO and Na_2O in varying proportions has been made. It is inferred that sodium zincate exists in concentrated solutions but that it is hydrolysed when the solutions are diluted, the zinc hydroxide set free as a consequence of hydrolysis exists in the colloidal state and when a critical dilution is reached it separates in the crystalline or amorphous form.

The behaviour of amphoteric oxides towards alkalis has been the theme of investigation of a large number of research workers but even to this day doubt exists concerning the formation of alkali salts of these oxides. In many cases attempts at the isolation of the alkali salts have given rise to conflicting data. Even the nature of solutions of these oxides in those of the hydroxides of the alkali metals is not clearly understood. The conclusions which may be drawn from the existing data are: (a) these solutions contain alkali salts of the amphoteric oxides, (b) they are colloidal solutions of the amphoteric oxides in alkalis, and (c) they contain the oxide in a colloidal form as well as an alkali salt. A systematic investigation of this problem has, therefore, been undertaken and a beginning is made with the study of the behaviour of zinc oxide towards sodium hydroxide.

The electrical conductivity measurements have been utilised for elucidating the condition of amphoteric oxides in solutions of sodium hydroxide or potassium hydroxide by a number of investigators but its use in the study of solutions of zinc oxide in those of sodium hydroxide has not received sufficient attention.

Hantzsch (*Z. anorg. Chem.*, 1902, **30**, 289) measured the electrical conductivity of solutions obtained by adding a solution of sodium hydroxide to that of zinc sulphate until the precipitated zinc hydroxide completely dissolved and concluded that in these solutions zinc hydroxide was present in the colloidal state. Subsequently he modified his views (*ibid.*, 1912, **76**, 371) and stated that the formation of sodium zinc oxides was not improbable and that these solutions might contain both the physical and chemical entities.

Carrara and Vespignani (*Gazzetta*, 1900, **30**, 63) and also Chatterjee and Dhar (*Trans. Faraday Soc.*, 1920, **16**, Appendix, p. 123) concluded from conductivity measurements that zinc hydroxide dissolved in caustic alkali

exists mainly as an alkali zincate. Snell (*J. Indian Chem. Soc.*, 1932, 9, 583) on the other hand inferred from the want of discontinuity in the equivalent conductivity ratio curves that no complexes are formed in these solutions. He has not given data at 30° which were obtained "with less satisfactorily regulated experimental conditions" and was unable to prepare solutions containing a higher ratio of ZnO : Na₂O than 0.4.

In the present investigation the electrical conductivity of solutions containing different amounts of zinc hydroxide and sodium hydroxide expressed as the ratios of ZnO and Na₂O has been measured at 30° between 9N and 1N for solutions containing a large proportion of sodium hydroxide and between 9N and 3N for solutions containing a large proportion of zinc oxide.

EXPERIMENTAL.

Preparation of Zinc Hydroxide.—Crystalline zinc hydroxide was prepared according to the method of Dietrich and Johnstone (*J. Amer. Chem. Soc.*, 1927, 49, 1419). These workers have not given sufficient details of the conditions of crystallisation and hence, after trial experiments, the following procedure was evolved which gave a yield of about 75%.

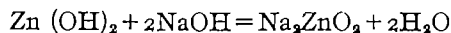
Zinc hydroxide was precipitated from recrystallised zinc sulphate (Merck's 'extra pure' quality) by ammonia, washed with hot water until free from sulphate ions and then dissolved in liquor ammonia (Merck's : d 0.888). The ammoniacal solution thus obtained was first kept under a bell jar together with a beaker containing three parts of concentrated sulphuric acid (d 1.84) and two parts of water. The concentration of the acid was increased the next day to four parts of the acid and one of water. This acid was kept for about 36 hours and was then replaced by concentrated sulphuric acid. After about four days crystals began to separate and within a week a good crop of crystals was obtained. These crystals were removed, washed and preserved after drying. The mother liquor deposited zinc hydroxide in the amorphous form but it was redissolved in ammonia and crystallised as before. The crystals were analysed for loss of water as well as for zinc and the results (H₂O, 18.1%; ZnO, 81.90%) showed the product to be pure zinc hydroxide.

Estimation of Zinc.—Zinc was estimated volumetrically using potassium ferrocyanide, standardised against a solution of zinc (free from iron) as well as of zinc oxide in sulphuric acid. In these titrations diphenylbenzidine was used as an internal indicator. It was prepared according to the method of Marquerol and Murror (*J. Chem. Soc.*, 1914, A i, 577) and dissolved in



concentrated sulphuric acid to give an 1% solution. The titrations were carried out as follows: A solution of sulphuric acid (3%) added to the solution containing zinc together with ammonium chloride (an excess of which was avoided as it retards the development of colour change) in order to obtain a sharp end-point and the whole was heated to 50-60° (cf. Kolthoff and Pearson, *Ind. Eng. Chem. Anal. Ed.*, 1932, 4, 147). Six drops of the indicator were then added and the solution of potassium ferrocyanide run in from a burette. At the end-point the colour is pale green.

Preparation of a Solution of Zinc Hydroxide in that of Sodium Hydroxide.—Preliminary experiments on the solubility of crystalline zinc hydroxide in solutions of sodium hydroxide showed that in low concentrations of the latter it was not appreciably soluble, but that a fairly large quantity of it dissolved in 10N sodium hydroxide. Calculated amount of crystalline zinc hydroxide required to form sodium zincate according to the equation



was added to a known volume of sodium hydroxide solution prepared by the method of Cornog (*J. Amer. Chem. Soc.*, 1921, 43, 2573). This solution was prepared in a Jena glass flask the contents of which were shaken vigorously on a mechanical shaker for about 10-12 hours. The solution was then warmed on a water-bath for about two hours and after cooling, it was filtered through a sintered Jena glass funnel. The clear filtrate was transferred to a Jena glass flask which was corked with a rubber stopper and kept in a desiccator over soda lime.

Method for Analysing the Zincate Solution.—The stock solution of sodium zincate prepared as described above was analysed to determine the ratio $\text{ZnO} : \text{Na}_2\text{O}$. The solution (1 or 2 c. c.) was measured by means of a micro-pipette in a standard flask and treated with a known volume of standard sulphuric acid (0.2N—0.3N) sufficient to keep the precipitated zinc hydroxide in solution. Zinc was estimated according to the method already described; sodium sulphate present in the solution does not interfere in this estimation as shown by Kolthoff and Pearson (*loc. cit.*). From the amount of zinc estimated the corresponding amount of ZnO was obtained by calculation.

For the determination of the Na_2O content of the solution the sodium hydroxide contained in it was determined as follows: From the total

quantity of sulphuric acid originally taken, the uncombined acid and that which combined with zinc to form zinc sulphate were subtracted and hence the amount of sulphuric acid equivalent to the sodium hydroxide present in the zincate solution was obtained. In order to determine the free sulphuric acid referred to above, a known volume of the zincate solution in sulphuric acid was titrated against a solution of ammonia (about 0.15N), standardised just before taking the reading with the test solution, using methyl red as an indicator. It is important to note that blank experiments with solutions containing known amounts of free sulphuric acid and zinc sulphate, when titrated by the above method, gave entirely satisfactory results.

Solutions containing ZnO and Na₂O in the proportions (i) 1:1.76, (ii) 1:2.44, (iii) 1:3.03, (iv) 1:3.55 and (v) 1:4.05 were prepared from the stock solution in the following manner. A known volume of the stock solution was taken and to this the calculated volume of sodium hydroxide (16N-18N) was added so as to give (very nearly) a desired ratio of ZnO:Na₂O. It was made up to a known volume with freshly redistilled water free from carbon dioxide. It was then analysed according to the method described above and preserved in a Jena glass flask closed with a rubber stopper and placed in an atmosphere free from carbon dioxide.

The Measurement of Electrical Conductivity.—Since the zincate solutions were strongly alkaline, a conductivity cell of resistant glass made in the form of a U-tube of capacity 3 to 4 c.c. was used. The platinum electrodes were fixed in position and coated with platinum black. The cell constant was sufficiently high being of the order of 25.35 and it was checked every time before and after taking a set of conductivity readings.

The electrical conductivities of all the solutions containing the different ratios were measured at 30°. The temperature was kept constant within $\pm 0.1^\circ$ by means of a thermostat controlled by a toluene regulator. Several readings for the resistance of a solution were taken and the conductivity was calculated from the mean value.

In the case of the ratio 1:1.76 only four dilutions were possible. In 4N dilution crystals, which on analysis were found to be those of zinc hydroxide, separated out after three hours and in the case of 3N dilution, separation of the crystals was observed even earlier. In dilutions of 2N and more, amorphous precipitate of zinc hydroxide separated out.

In 3N, 2N and 1.5N dilutions of the ratio 1:2.44 crystalline zinc hydroxide separated out on standing for a sufficiently long time but in lower

dilutions amorphous zinc hydroxide separated before a reading could be taken.

As the sodium hydroxide content of the solutions increased in proportion to the zinc hydroxide present, separation of crystalline or amorphous zinc hydroxide took place at much higher dilutions. But in no case it was possible to take conductivity readings of solutions below 1N as in every case, when a dilution of 0.5 N was prepared, zinc hydroxide appeared almost immediately.

The equivalent conductivities in reciprocal ohms of the different solutions used in this investigation are given in Table I.

TABLE I.

Ratios of ZnO : Na₂O in solutions

	1:1.76	1:2.44	1:3.03	1:3.55	1:4.05
9.41N*	13.14
9.20	...	18.49
9.50	20.37
9.48	23.10	...
9.47	24.54
8.0	21.23	26.18	29.30	32.75	35.36
6.0	38.45	47.19	48.60	53.37	55.98
4.0	63.50	73.70	78.70	83.80	86.40
3.0	79.92	91.50	101.2	104.8	108.2
2.0	...	117.9	121.2	134.8	136.5
1.5	...	129.95	138.2	149.3	152.3
1.0	149.8	173.1	176.9

DISCUSSION.

On a comparison of the data in Table I with those in Table II given below, in which the equivalent conductivity of sodium hydroxide at 30° obtained from the results of Bousfield and Lowry (*Phil. Trans.*, 1905, **A** 204, 205) is given, it will be found that the addition of zinc hydroxide diminishes the equivalent conductivity of sodium hydroxide.

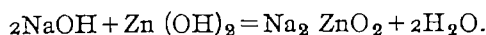
TABLE II.

Equivalent conductivity of sodium hydroxide at 30°.

N	...	10	8	6	4	3	2	1.5	1
Eq. conductivity	...	30.0	49.4	75.8	111.0	132.5	160.0	176.8	196.0

* N=Normality of the solution in terms of its NaOH content.

This decrease in the conductivity of sodium hydroxide may be explained on the disappearance of a part of the mobile hydroxyl ions due to the formation of sodium zincate according to the following reaction given for the simplest salt:



On this assumption it may be expected that, for a given concentration, an increase in the relative amount of zinc hydroxide from the ratio, 1 : 4.05 to 1 : 1.76 would give rise to more of sodium zincate leading to a diminution in the conductivity and this is in accord with the experimental results. But according to Hantzsch (*loc. cit.*) zinc hydroxide dissolved in sodium hydroxide exists in the colloidal state. On this postulate the disappearance of a part of the mobile hydroxyl ions may be explained as being due to their adsorption on the colloidal entities. An examination of the Tyndall cone in solutions of different concentrations showed that practically no cone was visible in 8N solution but that its intensity increased as the solution was diluted. It appears probable that sodium zincate exists in concentrated solutions but undergoes hydrolysis when they are diluted. The zinc hydroxide, set free as a consequence of hydrolysis, exists in the colloidal state and when the critical dilution is reached it separates in the crystalline or amorphous form. The increase in the equivalent conductivity with dilution for all solutions containing the different ratios of ZnO. Na₂O is to be attributed to the regeneration of the mobile hydroxyl ions as a result of the hydrolysis of sodium zincate on dilution.

When the equivalent conductivity is plotted against the ratio ZnO:Na₂O curves are obtained which are nearly linear and concave towards the ratio axis as observed by Snell (*loc. cit.*). The absence of a change in direction of these curves probably indicates that sodium zincate is present in all the solutions investigated. It appears that a change in direction of the ratio-equivalent conductivity curves is to be expected when there is a transition from a solution containing no salt to one in which a salt is formed or from a solution containing one salt to another containing a different salt. It is incorrect to conclude, as done by Snell (*loc. cit.*), that no complexes exist in these solutions just because of the absence of discontinuity in the ratio-equivalent conductivity curves.

CRYSTALLOGRAPHIC INVESTIGATION OF ARTOSTENONE,
THE STENONE ISOLATED FROM THE INDIAN SUM-
MER FRUIT, *ARTOCARPUS INTEGRIFOLIA*
BY MEANS OF GONIOMETER AND X-RAYS.

BY M. C. NATH AND P. L. MUKHERJEE.

Several morphological examinations have been made by means of the goniometer. The crystal system has been found to be monoclinic. Plate faces $a(100)$ of the crystals exhibit pronounced elongation along the c -axis. Crystallographic studies by means of X-rays gives $a = 17.3$, $b = 18.2$, $c = 7.4$ and $\beta = 100^\circ 49'$. The molecular weight as calculated from these results comes out to be 424.2 ($C_{30}H_{50}O$ requiring 426 as the molecular weight). The improbability of the presence of the CO group in the position C_3 , as in ergosterol, cholesterol etc., has been supported. The results supply additional support to the view that artostenone has got almost the same molecular structure as that of ergosterol.

The knowledge of the constitution of sterols, bile acids and related compounds has been revolutionised in the year 1932. The new conception first grew out of X-ray investigations by Bernal (*Nature*, 1932, **129**, 277, 721), who for the first time suggested that the then accepted formulae for sterols and bile acids, must be modified in order that they could be made to fit into the crystallographic cell. About three months after the publication of this paper, a new formula was suggested by Rosenheim and King (*Chem. Ind.*, 1932, **51**, 464, 954), which was later modified by Weiland and Dane (*Z. physiol. Chem.*, 1932, **210**, 268). This has been supported by crystallographic evidence (Bernal, *Chem. Ind.*, 1932, **51**, 466) and accepted universally. Crystal structure of several sex hormones from various sources, has also been determined recently with the help of X-rays (Bernal and Crowfoot, *Z. Krist.*, 1936, **98**, 464) and many valuable informations obtained.

It has been shown in previous papers (Nath, *Z. physiol. Chem.*, 1937, **247**, 9; 1937, **249**, 71, 76, 78), that artostenone resembles other sterols and stenones very closely, the only differences being in the position of CO group and the double bond.

Crystallographic investigation was undertaken with a view to determine the molecular structure and thus to obtain further evidence, if possible, of the close relationships, secured by chemical methods, between artostenone and the other known sterols and hormones.

Artostenone, when crystallised from a solution of alcohol-benzene-ethyl acetate mixture (3:2:1), forms fine crystals in the form of thick plates. These crystals have been examined both by the goniometer and X-rays. The preliminary results obtained are sufficient to give an idea as to the molecular weight, and shape and size of the complex artostenone molecule.

E X P E R I M E N T A L.

Goniometer Results

The plate faces $a(100)$, of the crystals, exhibit pronounced elongation along the c -axis. Several morphological examinations by the goniometer supply the following data.

TABLE I.

Crystal system	Monoclinic
Axial ratios as $a : b : c = 1.686 : 1.07306 : (1.683 : 1.07269 \text{ from X-ray data})$	
Axial angle $\beta = 100^\circ 49'$	
Observed forms $a(100)$, $c(001)$, $p(110)$ and $x(101)$	
Interfacial angles : $a : p = 58^\circ 52'$; $p : p = 62^\circ 18'$; $a : c = 79^\circ 11'$; $a : x = 75^\circ 57'$.	

Fig. 1 represents the structural sketch.

FIG 1

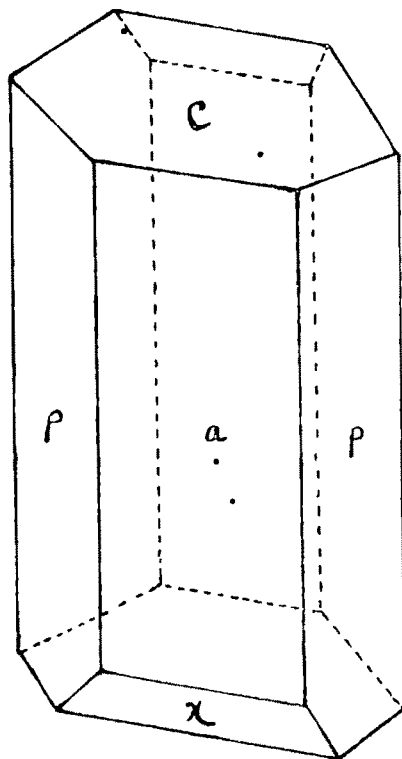
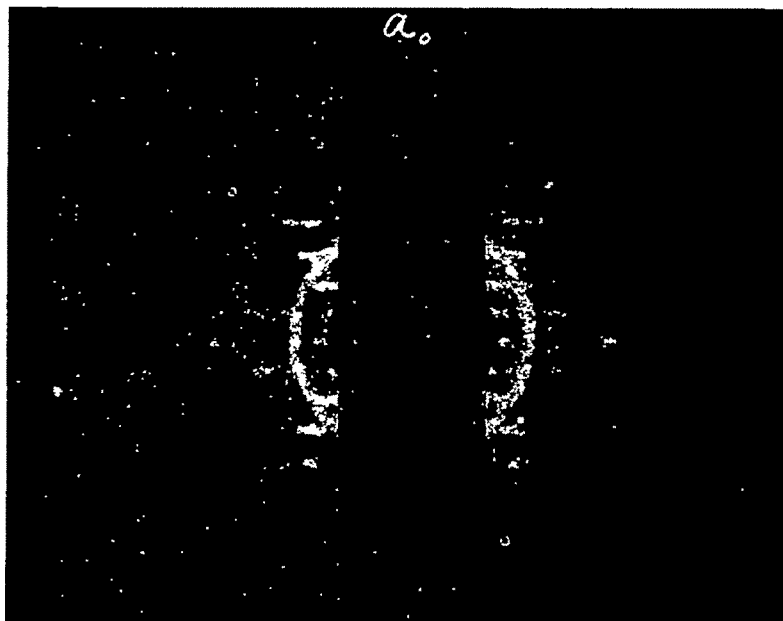


FIG. 2.

*X-ray Results.*

X-ray rotation photographs about the three principal crystal axes (as represented in Figures 2, 3 and 4) have been taken to determine the axial lengths and the size of the unit cell which comes out to be as follows

$$a_0 = 17.25, \quad b_0 = 10.25, \quad c_0 = 7.45 \quad \text{and} \quad \beta = 100^\circ 49'.$$

Density of the crystal has been found to be 1.083 by Retger's suspension method. Assuming the number of chemical molecules per unit cell to be two, the molecular weight comes out to be 424.2, which agrees well with the value (426) as determined by chemical method and required for the formula $C_{30}H_{50}O$, proposed for artostenone, in previous works (*loc. cit.*).

DISCUSSION.

Since there are two molecules in a unit cell, it can be assumed that the longest direction is along the *a*-axis, thickness along the *c*-axis and the *b*-axis is twice the breadth of the molecule.

FIG. 3.

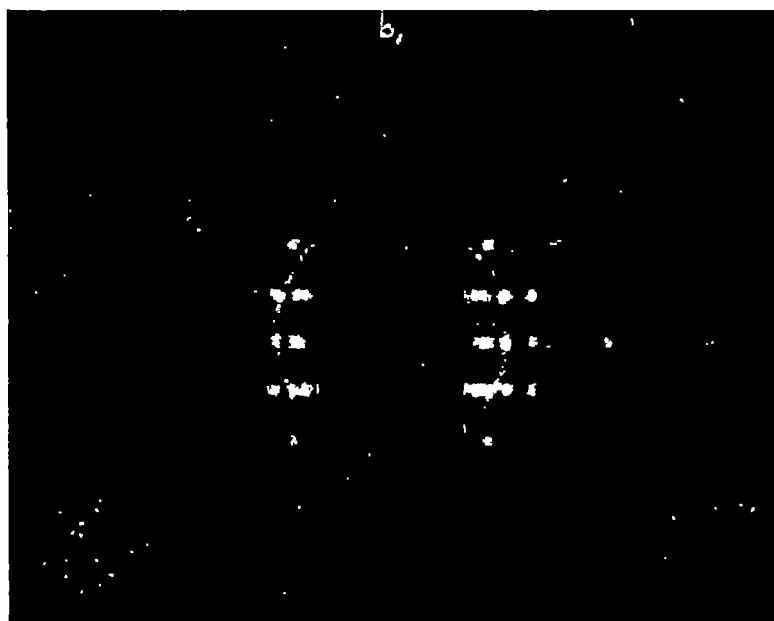
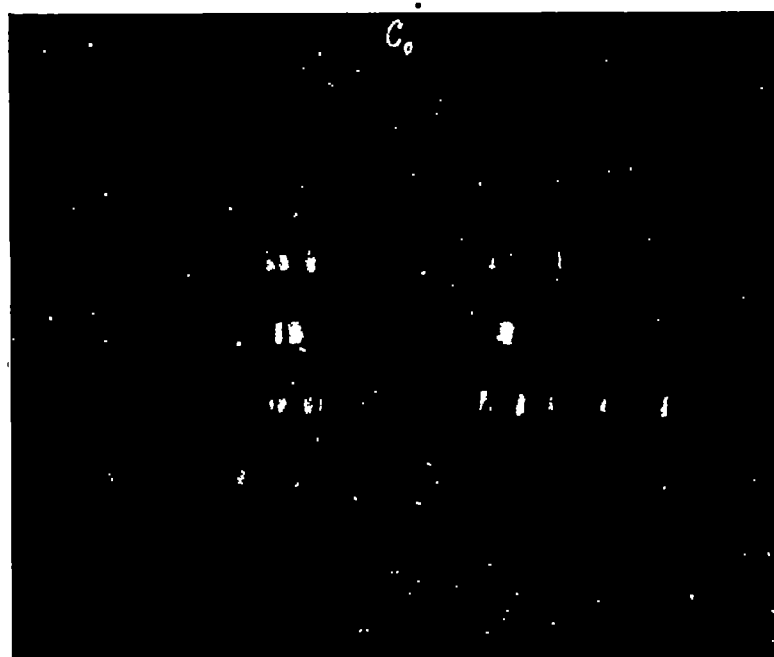


FIG. 4.

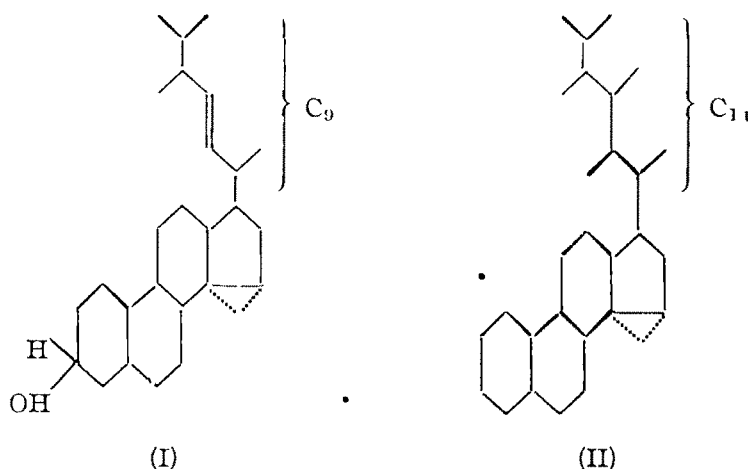


According to the models made to scale of the formula (I) of Rosenheim and King for ergosterol and its dimensions measured with due allowance for the space occupied by hydrogens, the results obtained are

Author's results $a = 4.5\text{\AA}$; $b = 7.4\text{\AA}$; $c = 20.0\text{\AA}$.

Bernal's results $a = 4.9\text{\AA}$; $b = 7.4\text{\AA}$, $c = 19.6\text{\AA}$

It has been shown in previous papers (*loc. cit.*) that in artostenone there is no OH group in structural formula (I) given below. Hence if the molecule is also assumed to be of the same model as (I), the length should be



shorter by about 1.5\AA . The other difference is that there is no double bond in the side-chain, but this should not change the dimensions appreciably.

Thus the molecular dimensions of artostenone as calculated from this cell comes out to be

$$a = 17.3\text{\AA}, \quad b = 7.4, \quad c = 5.1;$$

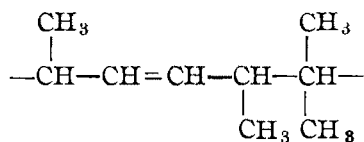
and those required by model (II) are

$$a = 4.9\text{\AA}, \quad b = 7.4 \quad \text{and} \quad c = 18.1$$

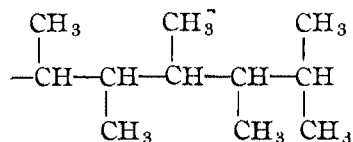
[as obtained after due allowance for the modifications stated above, from Bernal's values for ergosterol (*Nature*, 1932, **129**, 277)].

The agreement between the size of the artostenone molecule with that of ergosterol, is very close and that confirms very nicely the similarity between the structure of the carbon skeletons of these two compounds.

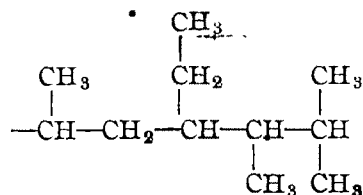
The result further settles conclusively another point regarding the structure of the side-chain. In case of ergosterol the chain has been found to be



An additional carbon atom beyond this chain in the linear direction will obviously necessitate a greater length for an artostenone molecule. If the chain in the artostenone molecule is assumed to be



or

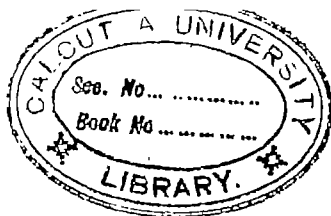


or something like this, the length of the molecule should be the same as that of ergosterol, and this has actually been observed in case of artostenone. Thus there are indications to show that artostenone, like ergosterol, also contains four condensed rings and a side-chain with five carbon atoms in the linear direction, other carbon atoms forming branches of the main line in some way or other.

Our best thanks are due to Prof. J. C. Ghosh and Prof. S. N. Bose for their kind and sympathetic interest and to Dr. K. Banerjee for his helpful advice and criticism in this work.

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Received April 6, 1939.



DISSOCIATION CONSTANTS OF SOME ORGANIC ACIDS FROM SOLUBILITY MEASUREMENTS.

BY W. V. BHAGWAT.

The dissociation constants of monochloro- and trichloro-acetic acids, aminobenzoic and propionic acids have been recorded

In previous papers (*J. Indian Chem. Soc.*, 1929, **6**, 207; 1933, **10**, 477) the dissociation constants of some inorganic acids have been determined and the limitations of the solubility method discussed. In this paper the work is continued on organic acids and the results with some of these are recorded below. In the tables *a* denotes solubility of the acids soluble in water; *b*, the solubility of the same in sodium salt of the acid of which the dissociation constant has to be determined; *c*, the original concentration of the salt in solution; k_2 , the dissociation constant of the unknown acid and k_1 , that of the known acid.

Dissociation Constant of Monochloroacetic Acid.

TABLE I.

With benzoic acid (k_1 at $29.6^\circ = 6.4 \times 10^{-5}$).

Temperature = 29.6° . $a = 0.03237$.

<i>c</i> .	<i>b</i> .	(<i>b</i> - <i>a</i>).	(<i>c</i> - <i>b</i> + <i>a</i>).	$k_2 = \frac{k_1 \times a (c - b + a)}{(b - a)^2}$.
0.01062	0.03433	0.00196	0.00866	4.67×10^{-3}
0.02083	0.03697	0.00360	0.01723	2.76
0.04923	0.03858	0.00621	0.04302	2.31
0.10830	0.04185	0.00948	0.09882	2.25
0.18050	0.04610	0.01373	0.16677	1.83
0.54160	0.05657	0.02420	0.51740	1.83

Temperature = 26.6° . $a = 0.02943$.

0.01321	0.03109	0.00196	0.01125	5.50×10^{-3}
0.02570	0.03289	0.00343	0.02227	3.57
0.06018	0.03597	0.00654	0.05364	2.36
0.27080	0.04479	0.01536	0.25544	2.12

Temperature = 25.4° . $a = 0.02763$.

0.10830	0.03662	0.00900	0.09930	2.17×10^{-3}
0.18050	0.03989	0.01226	0.16824	1.98
0.54160	0.05101	0.02338	0.51820	1.68

TABLE I (contd.)

With salicylic acid (k_1 at $25^\circ = 1.6 \times 10^{-3}$).Temperature = 25° $a = 0.01896$.

c	b	$(b-a)$	$(c-b+a)$	$k_2 = \frac{k_1 \times a(c-b+a)}{(b-a)^2}$
0.01320	0.02518	0.00622	0.06980	5.40×10^{-3}
0.02570	0.02845	0.00949	0.01621	6.83
0.03788	0.03237	0.01341	0.02447	3.26
0.04923	0.03466	0.01570	0.03353	4.10
0.06020	0.03760	0.01864	0.04156	3.60
0.08068	0.04186	0.02290	0.05778	3.32
0.10830	0.04611	0.02716	0.08115	3.32
0.14770	0.05346	0.03450	0.14320	2.87
0.27080	0.07290	0.05396	0.21680	2.24
0.54160	0.09810	0.07910	0.46250	2.20

Temperature = 28.2° $a = 0.01782$.

0.01320	0.02354	0.00572	0.00748	6.50×10^{-3}
0.03788	0.03106	0.01324	0.02464	4.00
0.09020	0.04185	0.02403	0.06617	3.27
0.18050	0.05673	0.03891	0.14160	2.67
0.54160	0.07701	0.05519	0.46460	2.18

Dissociation Constant of Trichloroacetic Acid.

TABLE II.

With benzoic acid (k_1 at $30^\circ = 6.4 \times 10^{-5}$)Temperature = 28.2° $a = 0.03057$

c	b	$(b-a)$	$(c-b+a)$	$k_2 = \frac{k_1 \times a(c-b+a)}{(b-a)^2}$
0.1741	0.0318	0.00131	0.1778	2.00×10^{-1}
0.2691	0.0327	0.00213	0.2670	1.15
0.5382	0.0368	0.00311	0.5351	1.07

Temperature = 30° $a = 0.03237$

0.5382	0.03400	0.00163	0.5366	4.03×10^{-1}
0.2691	0.03351	0.00114	0.2680	4.25
0.1076	0.03188	0.00065	0.1070	5.00
0.0490	0.03155	0.00027	0.0487	3.60

With salicylic acid (k_1 at $25^\circ = 1.6 \times 10^{-3}$).Temperature = 30.5° $a = 0.02125$

0.04900	0.02190	0.00075	0.0483	3.0×10^{-2}
0.01794	0.02240	0.00114	0.0168	2.10
0.05382	0.02422	0.00297	0.05082	2.10

Dissociation Constant of Aminobenzoic Acid.

TABLE III.

With benzoic acid (k_1 at $30^\circ = 6.4 \times 10^{-5}$).

Temperature = 28.2° $a = 0.03574$.

c	b	$(b-a)$	$(c-b+a)$	$k_2 = \frac{k_1 \times a(c-b+a)}{(b-a)^2}$
0.01090	0.03957	0.00900	0.00190	4.60×10^{-6}
0.01634	0.04447	0.01390	0.00244	2.47
0.03268	0.06049	0.02990	0.00276	3.31
0.04902	0.06294	0.03237	0.01665	3.12
0.09804	0.07848	0.04611	0.05193	4.78

With salicylic acid (k_1 at $24^\circ = 1.6 \times 10^{-3}$).

Temperature = 29.1° $a = 0.01847$

0.01090	0.02747	0.00900	0.00190	8.7×10^{-4}
0.01634	0.03433	0.01586	0.00375	4.4×10^{-4}
0.03268	0.03171	0.01325	0.01943	3.27×10^{-3}
0.04902	0.02877	0.01030	0.03872	1.07×10^{-2}
0.09804	0.02943	0.01096	0.08708	2.14×10^{-2}

Temperature = 28.3° $a = 0.01782$

0.00891	0.02518	0.00736	0.00155	8.1×10^{-4}
0.01090	0.02714	0.00932	0.00158	5.2×10^{-4}
0.01634	0.03221	0.01439	0.00522	7.2×10^{-4}
0.03268	0.03204	0.01422	0.01846	2.6×10^{-3}
0.04902	0.02812	0.01030	0.03872	1.04×10^{-2}
0.09804	0.02877	0.01095	0.08709	2.07×10^{-2}

Dissociation Constant of Propionic Acid.

TABLE IV.

*With benzoic acid (k_1 at $30^\circ = 6.4 \times 10^{-5}$).*Temperature = 28.1° $a = 0.03165$.

c .	b .	$(b-a)$.	$(c-b+a)$.	$k_2 = \frac{k_1 \times a(c-b+a)}{(b-a)^2}$.
0.02082	0.05035	0.01929	0.00153	8.18×10^{-6}
0.08130	0.09380	0.07832	0.00300	9.70×10^{-7}
0.17890	0.13758	0.10652	0.07240	1.17×10^{-5}
0.29830	0.19278	0.16172	0.13660	1.04
0.44740	0.23736	0.20630	0.24110	1.40
0.80480	0.30411	0.27305	0.62180	1.65

*With salicylic acid (k_1 at $25^\circ = 1.61 \times 10^{-3}$).*Temperature = 27.8° . $a = 0.01795$.

0.02082	0.03589	0.01794	0.00288	2.57×10^{-4}
0.08130	0.09264	0.07469	0.00660	3.40×10^{-5}
0.09940	0.10827	0.09032	0.00910	3.2
0.17890	0.17659	0.15860	0.02030	2.3
0.29830	0.27155	0.25360	0.04470	2.0
0.44740	0.37982	0.36180	0.08560	1.87
0.80480	0.73301	0.71500	0.17480	9.80×10^{-6}

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Received March 3, 1939.

RESONANCE REACTION PART II.

BY PANCHANAN NEOGI AND KANAI LAL MONDAL.

The formation of fumaric acid from maleic acid by resonance reaction has been completely established and the methyl ester characterised. The best yield of fumaric acid (50%) is obtained when maleic acid and MnO_2 exist in the proportion of 4 : 1. The conversion of citraconic acid to mesaconic acid by resonance reaction has been confirmed.

Neogi, Neogi and Chatterjee (*J. Indian Chem. Soc.*, 1928, **6**, 279) first showed that maleic acid was partly converted into fumaric acid when sulphur dioxide was passed through a solution of maleic acid in which manganese dioxide was suspended and that neither sulphur dioxide nor manganese dioxide alone as well as the products of the reaction could bring about the conversion. This was repeated by Neogi and Mitra (*J. Indian Chem. Soc.*, 1929, **6**, 969) who confirmed the result and named such a reaction 'resonance reaction.' Owing to the importance of the result, the reaction was studied (i) to confirm further the formation of fumaric acid by preparing its methyl ester, (ii) to ascertain the conditions under which the best yield of fumaric acid is obtained varying the proportions of manganese dioxide and maleic acid. The formation of fumaric acid has now been completely established and its methyl ester characterised. It has been shown here that the best yield of fumaric acid (50%) is obtained when the proportions of maleic acid and manganese dioxide are about 4 : 1.

Neogi and Mitra (*loc. cit.*) also studied the conversion of citraconic acid to mesaconic acid. This experiment has also been repeated and the result confirmed. The best yield of 20% crude and 10% pure mesaconic acid was obtained when the proportion of citraconic acid to manganese dioxide was also about 4 : 1. The yield of mesaconic acid from citraconic acid is, as already mentioned in the earlier paper, much less than that of fumaric from maleic acid.

EXPERIMENTAL.

Conversion of Maleic to Fumaric Acid.—To maleic acid (4 g.) dissolved in water (10 c.c.), a rapid current of sulphur dioxide was passed for about 5 minutes. As soon as manganese dioxide disappeared a white precipitate rapidly separated. This was filtered and crystallised from alcohol. About 1 g. of this substance was esterified with methyl alcohol (2 g.) and sulphuric acid (2 c.c.) by heating on a steam-bath for about 4 hours. The methyl ester after purification, had m.p. 100° , undepressed by an authentic specimen.

TABLE I.

Maleic acid.	MnO ₂ .	Duration of passage of SO ₂ .	Yield of fumaric acid.
4.0 g.	0.1 g.	5 min.	Nil
4	1.0	"	50%
4	2.5	Till MnO ₂ disappeared.	35%
4	4.0	Do	Very small.
2	4.0	Do	Do

Conversion of Citraconic Acid to Mesaconic Acid.—To a suspension of manganese dioxide (0.5 g.) in a solution of citraconic acid (2 g.) in water (10 c.c.) was passed sulphur dioxide till a clear solution resulted (3 or 4 minutes). The filtered solution was evaporated on the steam-bath and the residue extracted with ether, the ethereal layer being washed with equal volume of water. The removal of ether furnished pure mesaconic acid (*cf.* Franz, *Monatsh*, 1894, 15, 209), m.p. and mixed m.p. with an authentic specimen 202°. Mesaconic acid can be alternatively isolated by precipitation from the solution with ferric chloride as a brown compound whence after liberation with hydrochloric acid, it can be extracted out with ether. The acid isolated from the ether layer requires two crystallisations.

TABLE II.

Citraconic acid.	MnO ₂ .	Duration of passage of SO ₂	Yield.
2.0 g.	0.1 g.	Till solution	Nil
2	0.5	"	10%
2	1.0	"	8.5 (15% crude)
2.5	2.0	"	5%
2.0	5.0	"	Very small.

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Received April 6, 1939.

VITAMIN C AND TOXINS. PART III. THE EFFECT OF DIPHThERIA TOXIN ON VITAMIN C METABOLISM.

BY BALDIYANATH GHOSH.

A decrease in the ascorbic acid content of the blood, adrenal, liver and kidney of guinea-pigs was observed after the injection of 0.5 m.l.d of diphtheria toxin. During the period of intoxication a greater amount of ascorbic acid has been found to be excreted in the urine in a combined state.

We have previously observed that vitamin C *in vitro* has some specific inactivating effect on diphtheria toxin but that ingestion of massive doses of vitamin C can hardly confer any protection on experimental guinea-pigs when injected subsequently with 1 m.l.d. of diphtheria toxin (Ghosh and Guha, *J. Indian Chem. Soc.*, 1938, **16**, 438, 443). Zilva (*Brit. J. Expt. Path.*, 1937, **18**, 449) also found that guinea-pigs, when grouped as depleted, saturated or injected with ascorbic acid, behave practically identically to 1 m.l.d. of diphtheria toxin. King and Menten (*J. Nutrition*, 1935, **10**, 129, 141), however, have shown that when animals are partially depleted of their vitamin C reserves, their survival period after injection of diphtheria toxin is shortened about 50% and the loss in body-weight is more severe. Moreover, it was observed by various investigators (Lyman and King, *J. Pharmacol.*, 1936, **56**, 209; Philippe and Harde, *Compt. rend. soc. biol.*, 1936, **121**, 940; Torrance, *Proc. Soc. Expt Biol. Med.*, 1937, **35**, 654; Polonyi, *Wied. Med. Wochsch.*, 1935, **88**, 685; Haas, *Z. Immunitätsforsch.*, 1937, **91**, 203; Harris, Passmore and Pagel, *Lancet*, 1937, **233**, 183) that injection of diphtheria toxin in sublethal doses causes a depletion of vitamin C content of tissues of normally fed guinea-pigs. The diminution of the ascorbic acid in the adrenal or in other organs of guinea-pigs, indicates that during intoxication vitamin C metabolism is affected. Harris *et al.* (*J. Soc. Chem. Ind.*, 1936, **55**, 841) and others (Rinehart *et al.*, *Proc. Soc. Expt. Biol. Med.*, 1936, **35**, 347, 350) have observed that during fever and other conditions like rheumatism and tuberculosis, the vitamin C excretion in the urine is appreciably lowered and larger amounts of vitamin C require to be administered in order to maintain normal excretory level of vitamin C in the urine. Since the recent observations of Scarborough and Stewart (*Biochem. J.*, 1937, **31**, 2232) and the observations of Guha and Sen-Gupta (*Nature*, 1938, **141**, 974) indicate that in normal urine a certain amount of ascorbic acid is

excreted in a combined form, it was considered of interest to investigate whether during toxic condition a greater amount of ascorbic acid is excreted in the combined state, thereby reducing the amount of free ascorbic acid excreted. Thus ascorbic acid might serve as an internal detoxicating agent both in natural and infected conditions. A detailed investigation of the effect of diphtheria toxin (taken as an infective agent) on the ascorbic acid concentration of blood, tissues and urine of guinea-pigs was therefore, carried out in order to throw light on the fate of ascorbic acid during the period of intoxication.

EXPERIMENTAL.

Guinea-pigs were kept on a normal mixed diet of green grass and germinated gram. The animals, weighing between 280-350 g. were divided into several groups, each containing 5 animals. One group served as control while the other 4 groups were injected with 0.5 m.l.d. of standardised diphtheria toxin. Blood was drawn out from the heart of the guinea-pigs of these 4 groups 24, 48, 72 and 96 hours after toxin injection respectively, while the animals of the control group supplied figures for normal blood ascorbic acid. The ascorbic acid content was determined from 2 c.c. of oxalated blood by the usual titrimetric method (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935, **12**, 30) after precipitation of protein by trichloroacetic acid. The results are given in Table I.

TABLE I.

No. of expt.	Normal.	Mg. of ascorbic acid per 100 c.c. of blood.			
		Hours after 0.5 m.l.d. of diphtheria toxin injection.			
		24.	48.	72.	96.
1	0.64	0.65	0.47	0.30	0.50
2	0.65	0.74	0.54	0.43	0.37
3	0.76	0.43	0.47	0.40	0.52
4	0.65	0.40	0.50	0.40	0.44
5	0.65	0.40	0.55	0.43	0.35
Mean	0.67	0.52	0.50	0.39	0.43

It will be seen that there is a progressive decrease in the ascorbic acid value of the blood of guinea-pigs but there is a slight increase 3 days after toxin injection.

Similar experiments were carried out with other groups of guinea-pigs in order to estimate the vitamin C content of the adrenal, liver and kidney 24, 48, 72 and 96 hours after the injection of 0.5 m.l.d. of toxin (Table II). There is, it will be seen, about 50% decrease in the ascorbic acid content of the adrenal as compared with that of normals and there is also a definite reduction of the ascorbic acid content of the liver and kidney tissues after toxin injection.

TABLE II.

A. *Adrenal.*

Mg. of ascorbic acid per g. of adrenal.

No. of expt.	Normal	Hours after 0.5 m.l.d. of diphtheria toxin injection			
		24.	48.	72	96
1	0.348	0.220	0.216	0.172	0.180
2	0.366	0.277	0.200	0.170	0.200
3	0.366	0.290	0.360	0.195	0.215
4	0.330	0.300	0.277	0.225	0.161
5	0.290	0.305	0.340	0.205	0.191
Mean	0.340	0.278	0.278	0.193	0.189

B. *Liver.*

Mg. of ascorbic acid per 5 g. of liver.

No. of expt.	Normal.	Hours after 0.5 m.l.d. of diphtheria toxin injection.			
		24.	48.	72	96.
1	1.04	0.98	1.23	1.00	0.81
2	1.50	1.13	1.00	0.96	1.13
3	1.50	1.04	1.15	1.04	1.00
4	1.36	1.28	1.13	1.15	0.87
5	0.92	1.50	1.15	1.15	1.04
Mean	1.26	1.18	1.13	1.06	0.97

TABLE II (contd.).

C. *Kidney.*

Mg. of ascorbic acid per g. of kidney.

No of expt.	Normal	Hours after 0.5 ml d. of diphtheria toxin injection.			
		24.	48.	72.	96.
1	0.136	0.105	0.132	0.103	0.097
2	0.139	0.113	0.098	0.109	0.123
3	0.161	0.138	0.143	0.100	0.103
4	0.151	0.134	0.132	0.120	0.100
5	0.100	0.134	0.112	0.114	0.110
Mean	0.137	0.124	0.123	0.109	0.106

The estimation of ascorbic acid content (both free and combined) of the urine of guinea-pigs was carried out by the following method. Each animal was kept in a metabolism cage. The urine was collected over sufficient quantity of glacial acetic acid so that the final concentration of ascorbic acid remained near about 10% after washing the cage and funnel. Thiosulphate excreted in the urine was removed by addition of barium acetate (2 g.). This was centrifuged. From the supernatant liquid excess of barium was removed by addition of dilute sulphuric acid. This was centrifuged and was made up to a definite volume (50 c.c.) and stored in an amber coloured bottle. Into this urine H_2S was passed for 5 minutes and the bottle then well-corked. After $\frac{1}{2}$ hour an aliquot was taken out, H_2S was removed by a current of inert gas (CO_2 or N_2) and the vitamin C content was estimated by the usual method. A portion (5 c.c.) of this H_2S -free urine was brought to p_H 5.6, to which 2 c.c. of acetate buffer (p_H 5.6) and 1 c.c. of ascorbic acid oxidase solution (prepared freshly from pressed cucumber juice) were added. The mixture was well shaken and kept at 40° for $\frac{1}{2}$ hour. The contents were made up to a definite volume (10 c.c.) and the reducing substances present were estimated. The difference between the values obtained before and after oxidase treatment gives the true ascorbic acid value (*cf.* Sen-Gupta and Guha, *Science and Culture*, 1938, 8, 398). The remaining urine solution was kept in H_2S atmosphere for a period of 72 hours after which an aliquot was taken out and similarly its true vitamin C content was estimated. After 96 hours of reduction in H_2S atmosphere the remaining portion of the urine was likewise treated. The maximum value of true ascorbic acid is obtained between 72 and 96 hours of reduction in H_2S atmosphere. For present purpose the difference

between the initial value and the maximum value obtained after H_2S treatment has been taken to be the amount of ascorbic acid present in combined state (*cf.* Scarborough and Stewart, *loc. cit.*). *

In the present set of experiments each guinea-pig was employed as a self-controlled animal. The urine was estimated for 2 or 3 days before and after 0.5 m. l. d. of diphtheria toxin injection. The results (Table III) show that in almost all cases there is a decrease in free ascorbic acid excretion and an increase in the combined ascorbic acid (released after H_2S -treatment) after intoxication. There is, of course, no quantitative correlation between the diminution of urinary ascorbic acid and increase in ascorbigen following intoxication, as even under normal circumstances the urinary excretion of ascorbic acid and ascorbigen vary considerably from day to day. Also the extra ascorbigen in urine in some cases may account for some of the loss of ascorbic acid from the tissues.

TABLE III.

Mg. of ascorbic acid excreted in the urine during 24 hours per guinea-pig.

No. of expt.	No. of days.	Free ascorbic acid.		Combined ascorbic acid in terms of ascorbic acid.	
		Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection
I.	1	0.387	0.299	0.279	0.317
	2	0.398	0.277	0.215	0.253
	3	0.337	0.148	0.313	0.371
	Mean	0.374	0.241	0.269	0.313
II.	1	0.330	0.256	0.231	0.463
	2	0.271	0.270	0.241	0.452
	3	0.261	0.192	0.224	0.248
	Mean	0.287	0.239	0.232	0.387
III.	1	0.387	0.291	0.275	0.449
	2	0.330	0.286	0.324	0.461
	3	0.257	0.292	0.443	0.362
	Mean	0.324	0.289	0.347	0.424

* This question, however, has not yet been settled and further work is in progress.

TABLE III (contd.).

IV.	1	0.300	0.360	0.337	0.256
	2	0.377	0.249	0.313	0.429
	Mean	0.338	0.304	0.325	0.342
V	1	0.310	0.310	0.325	0.365
	2	0.415	0.360	0.337	0.498
	Mean	0.362	0.335	0.331	0.431
VI.	1	0.375	0.233	0.345	0.417
	2	0.325	0.200	0.202	0.415
	Mean	0.350	0.216	0.273	0.416
VII.	1	0.520	0.375	0.170	0.425
	2	0.390	0.357	0.107	0.391
	Mean	0.455	0.366	0.138	0.408

C O N C L U S I O N.

A decrease in the ascorbic acid content of the blood of normally fed guinea-pigs was observed on the 2nd, 3rd and 4th days after the injection of 0.5 m. l. d. of diphtheria toxin.

Similar injection of 0.5 m. l. d. of diphtheria toxin causes a diminution of the ascorbic acid content of adrenal, liver and kidney of guinea pigs. The loss in the adrenal gland is about 50%.

During the period of intoxication a greater amount of ascorbic acid is excreted in the urine in a combined state and a smaller amount in the free state. The combined ascorbic acid excreted may account for the loss of free ascorbic acid in the urine and also for the loss in the tissues during infection.

My best thanks are due to Prof B. C. Guha for his advice and interest. We are grateful to the Indian Research Fund Association for financing these researches.

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Received March 15, 1939.

ACTION OF FUMING NITRIC ACID ON IODINE.

By R. K. BAHL AND SURJIT SINGH.

Iodine pentoxide is the product obtained by the interaction of iodine and fuming nitric acid, under ordinary conditions. By removing the oxides of nitrogen from the original yellow powder, in the absence of moisture, iodine dioxide is also formed.

Millon (*Ann. chim. phys.*, 1844, **12**, 330, 345, 353; *J. pr. Chem.*, 1845, **34**, 321) has described the preparation of iodine dioxide by the action of very concentrated nitric acid on iodine. Bahl and Partington (*J. Chem. Soc.*, 1935, 1258) have, however, found that iodine when reacted upon by nitric acid (*d 1.5*) gives iodine pentoxide, a result which agrees with that of Guichard (*Compt. rend.*, 1909, **148**, 1923, *Bull. Soc. chim.*, 1909, **5**, 86) who obtained a better yield by using nitric anhydride.

Moles and Villan (*Anal. Fis. Quim.*, 1936, **34**, 787) found that the product of this reaction was iodine dioxide, I_2O_4 , mixed with free nitric acid.

These conflicting observations have necessitated a revision of this investigation of the action of nitric acid on iodine. It has been found, as stated by Bahl and Partington (*loc. cit.*), that by triturating iodine with fuming nitric acid and drying the product under ordinary conditions, iodine pentoxide is obtained. Analytical results are given in the experimental part.

EXPERIMENTAL.

Iodine was determined by the reduction with sulphur dioxide and precipitation of silver iodide in the presence of nitric acid. Oxygen was estimated as described by Bahl and Partington (*J. Chem. Soc.*, 1934, 1088).

TABLE I.

Sample.	Iodine.	Oxygen.
A	75.98 %	23.70 %
B	75.50	23.80
C	75.52	23.65

These results show that the product is mainly iodine pentoxide, I_2O_5 (Calc. I, 76.04%; O, 23.96%).

By modifying the conditions of the experiment it was, however, found possible to prepare appreciable quantities of iodine dioxide, I_2O_4 . This was done by removing the oxides of nitrogen by passing dry air through the iodine oxide contained in a glass Gooch sinter. The last traces of the nitrogen oxides were absorbed by solid caustic potash in a desiccator. The atmosphere in the latter was kept dry by placing some phosphorus pentoxide inside it. Low temperature gives a better yield, so the desiccator was kept cool in the ice-cold water. The dry product thus obtained was washed with water, alcohol and ether respectively over a suction pump. It was finally dried over phosphorus pentoxide in a desiccator. The product was analysed as before and the results are recorded below.

TABLE II.

Sample.	Iodine.	Oxygen.
A	79.50 %	20.20 %
B	79.29	20.09
C	79.40	20.16
D	79.17	20.02

(I_2O_4 requires I, 79.88% ; O, 20.12%).

It was noticed that the yellow voluminous powder, obtained by triturating iodine with fuming nitric acid, decomposed on exposure to give the brown iodine pentoxide. This decomposition is brought about by two factors, viz., (i) heat and (ii) moisture.

The latter factor had a greater influence on the decomposition of iodine dioxide in the presence of the fumes of the oxides of nitrogen due to the generation of heat, produced by the absorption of moisture by the fumes of the oxides of nitrogen. This view was confirmed by the addition of a drop of water to a freshly prepared yellow powder before removal of the oxides of nitrogen, when the whole mass rapidly turned brown.

This decomposition can, to a very great extent, be prevented by removing the oxides of nitrogen in complete absence of moisture.

It is clear, therefore, that the primary product of the interaction of iodine and fuming nitric acid is iodine dioxide, I_2O_4 , which in contact with moisture decomposes into iodine pentoxide and iodine, which colours the product brown.

ADSORPTIVE PROPERTIES OF SYNTHETIC RESINS. PART II. ADSORPTION OF POTASSIUM SALTS OF VARIOUS ANIONS.

BY S. S. BHATNAGAR, A. N. KAPUR AND MAHENDRA SARUP
BHATNAGAR.

The adsorption of about a dozen potassium salts of various anions by an amino-resin has been studied. In similar anions the adsorption decreases with increase in molecular weight. The same order of adsorption has also been observed for homologous series of mono- and dicarboxy fatty acids.

Anti-batic solubility-adsorbability relationship is not the sole determining factor. Consideration has to be paid to the molecular weight of the adsorbate and the possibility of its accomodation in the capillary structure of the adsorbent.

Adams and Holmes (*J. Soc. Chem. Ind.*, 1935, **54**, 11) were the first to study the adsorptive properties of the synthetic resins though S. Sato and Sekine (*J. Soc. Chem. Ind. Japan*, 1921, **24**, 51) had drawn attention to the amphoteric nature of the phenol-formaldehyde resins and Shono (*ibid.*, 1926, **29**, 53) had recorded the behaviour of some forty metallic salts which reacted with phenolic resins to give coloured derivatives. Adams and Holmes (*loc. cit.*) allowed the solution under examination to run down a column packed with the resin, and, although difficulties due to channelling made the results more or less qualitative, polyhydric phenol-resins were found to exhibit strong and selective adsorptive properties for a large number of cations and amino-resins were found to be quite efficacious for the removal of anions from solution. The consecutive use of phenolic and amino-resins was suggested to effect complete removal of dissolved salts from solution. In a previous paper Bhatnagar, Kapur and Puri (*J. Indian Chem. Soc.*, 1936, **13**, 679) from a quantitative study of the adsorption of a large number of organic and inorganic acids and bases, concluded that the removal of substances from solution by synthetic resins follows the ordinary laws of adsorption. In the present paper the authors have studied the adsorption of a number of potassium salts of various anions by an amino-resin.

The adsorption of anions on acid-extracted charcoal has been studied by Oden and Langelius (*J. Phys. Chem.*, 1921, **25**, 311). They found that the order of adsorption for various ions changed with concentration. Thus at 0.01M concentration the order of adsorption was $I' > CrO_4'' > CNS' > Fe(CN)_6''' > Br' > ClO_3' > Cl'$ and at 0.2M concentration $CNS' > I' > ClO_3' > CrO_4'' > Br' \geq Cl' > Fe(CN)_6'''$. The order $I' \geq Br' \geq Cl'$ has

also been confirmed by Schilov and Chepelevetskii (*Z. physikal. Chem.*, 1926, **123**, 248) by use of blood charcoal and activated sugar charcoal.

Beckley and Taylor (*J. Phys. Chem.*, 1925, **29**, 942) have studied the adsorption of silver salts on silver iodide; Mukerjee and Ray (*J. Indian Chem. Soc.*, 1924, **1**, 173) of potassium salts on lead chromate; Dhar and co-workers (*Kolloid Z.*, 1924, **35**, 144) of sodium and potassium salts on freshly precipitated barium sulphate, sols of aluminium hydroxide and vanadium pentoxide. Though various theories have been put forward to explain the adsorption on considerations of valency, coagulating power and solubility of the salts in water, no successful explanation has so far been given which could explain all the facts. In the present paper the authors have come to the conclusion that though there is a general relation between adsorbability and solubility, consideration should also be paid to the molecular weight of the anion.

EXPERIMENTAL.

The resin was prepared by dissolving two parts of *m*-phenylenediamine in 10 parts of dilute hydrochloric acid (1 : 1) to which 5 parts of 40% solution of formaldehyde were slowly added with constant stirring. A dark chocolate coloured resin was immediately precipitated. It was dried in presence of hydrochloric acid to render it completely insoluble in water. It was then repeatedly washed with hot water to remove all soluble impurities, dried, ground to a fine powder and passed through a 100 mesh sieve. A large sample of the resin was prepared in the above manner and used throughout all studies. Thus variations of the interface were only those existing through the mass of the adsorbent. The resin on ignition left no ash.

It should be mentioned here that all attempts to produce water-insoluble resins by condensation in presence of alkalis failed, a soluble product being obtained in every case.

The adsorption experiments were carried out by weighing exactly 1 g. of the resin in clean, dry, glass bottles of the same size to which 100 c.c. of the solution were pipetted. A blank experiment was carried on side by side. The bottles were shaken for the same length of time and kept for thirty-six hours, at the end of which interval the supernatant liquid was decanted and the amount of the solute determined by usual analytical methods. The blank experiment gave the original concentration (C_0), that with the resin the equilibrium concentration (C_e). The amount of adsorption (x) was determined by difference. Equilibrium concentrations were calculated to moles per litre.

The results for adsorption of various anions are recorded in Tables I, IV and V.

RESULTS AND DISCUSSION.

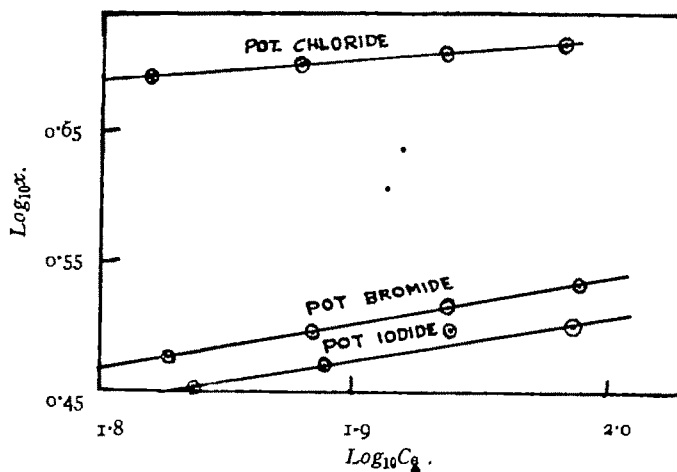
TABLE I.

	C_0	C_e	x	$\log C_e$	$\log x$	% Adsorption.	Solubility (g / 100 cc. of water at 20°).
KCl	10.000	9.475	0.525	0.9765	1.7202	5.25	34.0
	9.028	8.511	0.517	0.9300	1.7235	5.73	
	8.007	7.500	0.507	0.8751	1.7050	6.33	
	7.000	6.503	0.497	0.8131	1.6964	7.10	
KBr	10.000	9.654	0.346	0.9347	1.5391	3.46	65.2
	8.941	8.610	0.331	0.9350	1.5198	3.81	
	7.940	7.624	0.316	0.8822	1.4997	3.98	
	7.000	6.700	0.300	0.8261	1.4771	4.49	
KI	10.000	9.582	0.318	0.9860	1.5024	3.18	144.0
	9.000	8.686	0.314	0.9388	1.4969	3.49	
	8.000	7.704	0.296	0.8867	1.4713	3.70	
	7.034	6.750	0.284	0.8293	1.4533	4.04	

The adsorption curves are traced graphically and shown in Figs. 1-5. If we consider the various simple halogen salts (Table I), it appears

FIG. 1.

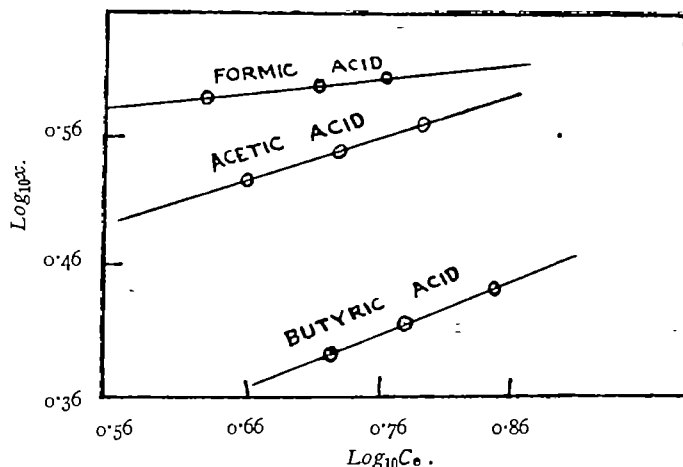
Adsorption of simple anions by 1 g. of resin from 100 c.c. of the aq. soln.



clearly that, with anions of increasing atomic weight, a marked decrease in the adsorbed quantity is obtained. This order is exactly reverse to that

FIG 2.

Sorption of monocarboxy fatty acids by 1 g. of the resin from 100 c.c. of the aq. soln.



obtained by Oden and Langelius (*loc. cit.*) and others on charcoal who found that $I' > Br' > Cl'$. This is as was to be expected, for we have found that in basic resins the normal Traube's rule is also reversed for a homologous series of fatty acids. The results for the adsorption of mono- and dicarboxy fatty acids are given in Tables II and III. It will be seen that the order of adsorption: formic > acetic > butyric and oxalic > malonic > succinic > adipic shows that the adsorption decreases with the increase in molecular weight, which is quite in line with the results obtained in the case of similar anions.

TABLE II.

Monocarboxy fatty acids.

Sorption by 1 g. of the resin from 100 c.c. of aqueous solution of acids.

Acids.	C_a .	C_s .	x .	$\text{Log } C_a$.	$\text{Log } x$.	% Sorption.
Formic	10.050	5.981	4.069	0.7768	0.6095	40.47
	9.346	5.320	4.026	0.7259	0.6048	43.00
	8.223	4.299	3.924	0.6334	0.5937	47.73
	7.476	3.645	3.831	0.5817	0.5833	51.25
Acetic	10.090	6.354	3.736	0.8031	0.5724	37.04
	9.030	5.467	3.563	0.7378	0.5518	39.45
	8.037	4.673	3.364	0.6696	0.5268	41.85
	7.010	3.832	3.178	0.5834	0.5022	45.33
Butyric	10.000	7.196	2.804	0.8571	0.4478	28.04
	8.738	6.120	2.618	0.7868	0.4179	30.00
	7.943	5.467	2.476	0.7378	0.3938	31.17
	7.010	4.673	2.337	0.6696	0.3687	33.33

TABLE III.

Dicarboxy fatty acids

Sorption by 1 g. of the resin from 100 c.c. of the aqueous solution of acids.

Acid.	C_0	C_e	x	$\text{Log } C_e$	$\text{Log } x$	% Sorption.
Oxalic	9.9060	1.1215	8.7845	0.0498	0.9437	88.68
	8.9730	0.9346	8.0384	1.9706	0.9051	89.58
	7.8500	0.7476	7.1024	1.8737	0.8515	90.48
	7.0100	0.6074	6.4026	1.7835	0.8069	91.33
Malonic	10.090	1.402	8.688	0.1467	0.9389	86.11
	8.925	1.028	7.897	0.0120	0.8975	88.48
	8.409	0.8879	7.5211	1.9483	0.8763	89.44
	7.523	0.6541	0.8689	1.8157	0.8369	91.30
Succinic	11.400	2.990	8.410	0.4757	0.9248	73.77
	9.438	2.336	7.102	0.3685	0.8514	75.25
	8.223	1.865	6.358	0.2716	0.8033	77.27
	7.290	1.588	5.702	0.2010	0.7561	78.20
Adipic	10.230	3.983	7.147	0.4891	0.8541	70.14
	9.159	2.523	6.636	0.4020	0.8219	72.45
	8.178	2.055	6.123	0.3130	0.7870	74.86
	7.296	1.682	5.608	0.2259	0.7488	76.92

Further, it is interesting to note from Table I that the order of adsorption is exactly the reverse order of the solubilities of the salts in water. This result is confirmed by the observations of Beckley and Taylor (*loc. cit.*) and Mukhrjee and Ray (*loc. cit.*) who have also found that in general the less soluble salts are sorbed more than the soluble ones.

Coming to the monovalent complex anions (Table IV) we see that the inverse solubility-absorption relation does not hold in quite an exact manner.

TABLE IV.

	C_o	C_e	x	$\text{Log } C_e$	$\text{Log } x$	% Adsorption	Solubility (g / 100 c.c. of water at 20°.)
KClO ₃	12'600	9'300	3'300	0'9685	0'5185	26'19	7'4
	9'150	6'525	2'625	0'8145	0'4149	28'69	
	7'725	5'400	2'325	0'7324	0'3664	30'97	
	6'000	4'050	1'950	0'6075	0'2900	32'50	
KBrO ₃	10'000	8'000	2'000	0'9031	0'3010	20'00	6'9
	9'022	7'160	1'862	0'8549	0'2700	20'64	
	8'030	6'310	1'720	0'8000	0'2355	21'42	
	7'270	5'650	1'620	0'7520	0'2095	22'28	
KIO ₃	9'733	8'398	1'335	0'9242	0'1255	13'72	8'13
	9'357	8'067	1'290	0'9067	0'1106	13'79	
	8'400	7'200	1'200	0'8573	0'0792	14'29	
	6'500	5'498	1'010	0'7396	0'0043	15'54	
KMnO ₄	10'000	1'919	8'081	0'2830	0'9075	80'81	6'34
	9'260	1'650	7'610	0'2175	0'8814	82'17	
	8'020	1'250	6'770	0'0969	0'8306	84'40	
	6'616	0'818	5'798	0'9128	0'7633	87'63	
KCNS	10'000	9'745	0'255	0'9888	1'4065	2'55	68'5
	9'000	8'757	0'243	0'9423	1'3856	2'70	
	7'975	7'745	0'230	0'8890	1'3617	2'88	
	6'918	6'700	0'218	0'8261	1'3385	3'15	

It should be noted that while there is a general relation between the solubility and the adsorbability of a solute there are still notable exceptions, *e.g.*, sulphanilic acid though less soluble than benzene sulphonc acid is less

FIG. 3.

Sorption of dicarboxy fatty acids by 1 g. of the resin from 100 c.c. of the aq. soln.

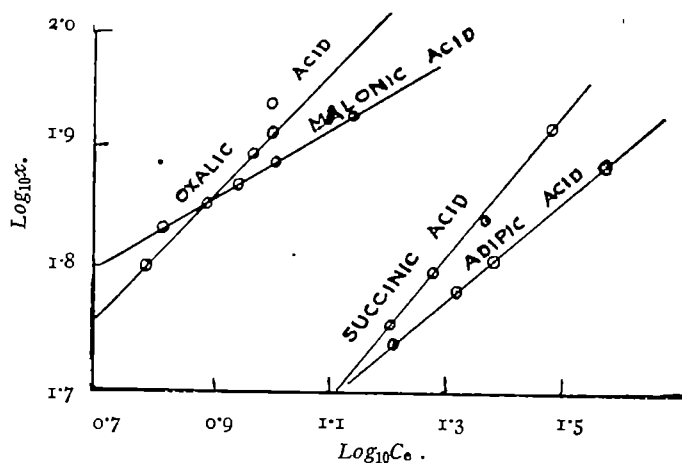
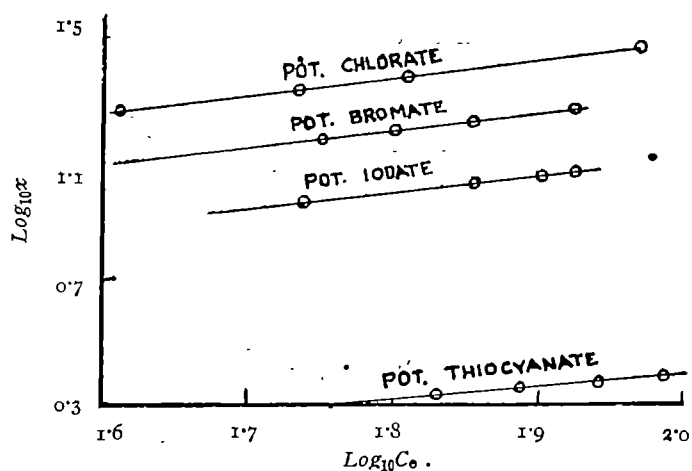


FIG. 4.

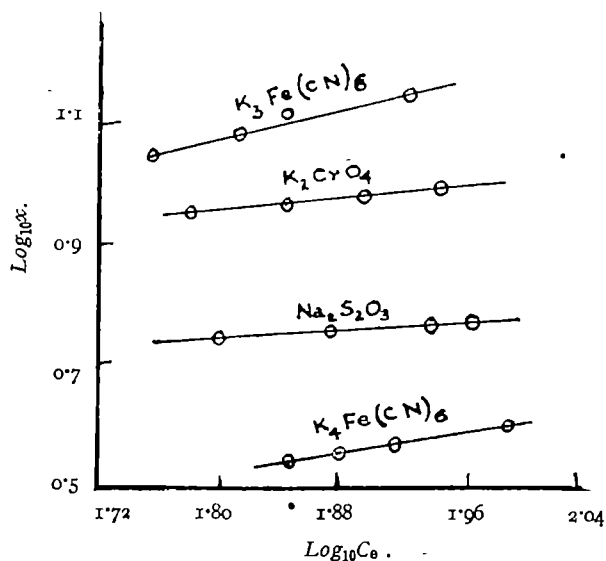
Sorption of complex monovalent anions by 1 g. of resin from 100 c.c. of the aq. soln.



strongly adsorbed. Thus low solubility alone is not sufficient to determine adsorbability, it being also necessary to consider if the anion can be accommodated in the internal capillary structure of the adsorbent. Sulphanilic acid having greater molecular weight is adsorbed less than benzene sulphonic acid. Similarly in the case of chlorate, bromate, iodate, though bromate is less soluble than the chlorate and should have been expected to be adsorbed more strongly, is adsorbed less owing to its greater atomic

FIG. 5.

Absorption of complex polyvalent anions by 1 g. of the resin from 100 c.c. of the aq. soln



weight. This argument receives confirmation from the work of Hahn (Be1., 1926, 89, 2014) who found that in the case of radioactive elements the anti-batic solubility-adsorbability relationship is not the sole determining factor but it has to be considered if the element can be incorporated in the crystal lattice of the sparingly soluble precipitate.

The abnormally high adsorption of potassium permanganate is explainable on the fact that it reacts chemically with the resin owing to its strong oxidising nature.

TABLE V.

C_s	C_a	x_s	$\text{Log } C_s$	$\text{Log } x_s$	% Adsorption.	Solubility (in g./100 c.c. of water at 20°).
$\text{K}_2\text{Cr}_2\text{O}_7$	10.000	6.019	3.981	0.7795	0.6000	39.81
	9.031	5.400	3.631	0.7324	0.5600	40.20
	8.000	4.760	3.241	0.6776	0.5105	40.50
	7.000	4.140	2.860	0.6170	0.4564	40.86

TABLE V (contd.).

K_2CrO_4	10'000	9'000	1 000	0 9542	0'0000	10'00	61 7
	8'965	8'000	0 965	0 9031	1'9845	10'76	
	8 000	7'066	0'934	0'8492	1 9703	11'68	
	7 000	6'100	0'900	0 7853	1'9542	12 85	
$Na_2S_2O_3$	10'000	9'408	0'592	0 9735	1'7723	5'92	70'07
	9 501	8'912	0 589	0 9500	1 7701	6'20	
	8'160	7 585	0'575	0 8800	1'7597	7 04	
	6'930	6'370	0'560	0'8041	1 7482	8 08	
$K_3[Fe(CN)_6]$	10'000	8'571	1 429	0'9331	0'1550	14 29	43'0
	8'400	7 071	1 329	0'8495	0 1235	15'82	
	7 810	6'570	1'240	0'8176	0 0934	16'00	
	6'857	5'714	1'143	0'7569	0'0580	16 67	
$K_4[Fe(CN)_6]$	10'450	10'050	0 400	1 0021	1 6021	3'83	32 97
	8 772	8'400	0'372	0'9243	1'5705	4'25	
	8'040	7'680	0 360	0 8854	1 5563	4 45	
	7'350	7 000	0'350	0 8513	1 5441	4'76	

Table V shows the adsorption of some bi-, tri- and tetra-valent anions. We have not as yet tried sufficiently large number of anions to draw any generalisation, but from the little that we have, it is obvious that there is no relation between valency and adsorbability. It should be observed, however, that among the bivalent anions $Cr_2O_7^{2-}$, CrO_4^{2-} , $S_2O_3^{2-}$, the order of adsorbability is $Cr_2O_7^{2-} > CrO_4^{2-} > S_2O_3^{2-}$ which is exactly the reverse of their solubility.

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Received May 5, 1939

A NOTE ON THE BASICITY AND MOLECULAR WEIGHT OF SHELLAC.

BY S. R. PALIT AND G. N. BHATTACHARYA.

The basicity and the molecular weight of the two main constituent resin acids of shellac, *viz.*, the pure lac resin and the soft resin of lac, have been ascertained from a study of their behaviour with caustic alkali. An acid potassium salt of the pure lac resin has been isolated by partial neutralisation of the resin with caustic potash and its properties studied.

A knowledge of the basicity and molecular weight of the constituent acids in shellac is an important step towards the study of its constitution. Gardner and his co-workers (*Ind. Eng. Chem.*, 1933, **25**, 696) have found the molecular weight of bleached shellac to be about 1000, whilst work at this Institute (*Ann. Rep. Indian Lac. Res. Inst.*, 1937-38, p. 3; Bhattacharya and Sen, *Proc. Indian Sci. Cong.* Part III, 1939) indicated that the ether-soluble soft resin and the insoluble pure lac resin have molecular weights of about 500 and 1900 respectively.

When an alcoholic solution of dewaxed shellac is treated with only 70% of KOH required to completely neutralise it, the latter remains clear on dilution with water. This shows that the solution contains no free shellac, which is insoluble in water, but contains a water-soluble acid potassium salt, which could be readily isolated from the solution. This observation is contrary to the view of Weinberger and Gardner (*Ind. Eng. Chem. Anal. Ed.*, 1933, **5**, 267) who stated that clear aqueous solutions are obtained only when sufficient alcoholic alkali is added to correspond to the acid number.

On further investigation with both pure and soft resin, it was found that the above observation is only true of pure lac resin but not of soft resin, which is precipitated from the alcoholic solution on addition of water unless completely neutralised. The alkali clarification value of pure lac resin, however, is 60% of the alkali required for complete neutralisation. This suggests that the basicity of pure resin in shellac is more than one. The acid potassium salt of pure lac resin was precipitated by slowly adding a saturated potassium chloride solution to a 20% alcoholic solution of pure lac resin, treated with sufficient aqueous caustic potash (0.5N) solution (about 60% of the total acid value)

and then diluted to about eight times with water. As the acid salt dissolves very slowly in cold water, it could be repeatedly washed with cold water until free from chloride and was then dried in a vacuum desiccator. It is a white powder, which turns slightly yellow on complete dehydration and is readily soluble in warm water forming a clear solution. Its acid value was found to be 29.0 which is half the acid value of the desiccated pure lac resin (58.4). The potassium content of the acid salt was found by analysis to be 28.76 (expressed in mg. of caustic potash per g. of the salt). It is concluded, therefore, that pure lac resin is dibasic. Similar conclusions were arrived at for bleached shellac from molecular weight and acid value considerations by Gardner and his co-workers (*loc. cit.*).

The amount of alkali required for the formation of the acid salt of a dibasic acid should be 50% of its acid value, but shellac acids being very weak and insoluble in water, a distinct excess of alkali (about 10% of the total acid value) is required to drive back the hydrolysis of the acid salt and to prevent the precipitation of free shellac on dilution with water. The observed effect of alkali on pure lac resin may be attributed at first sight to some sort of peptising action. But the acid value and the potassium content of the acid salt speak against such a view. Further pure resin dissolves in the aqueous normal potassium salt solution forming the same acid salt. This fact also reveals the dibasic character of the pure resin.

Though undoubtedly there is some colloidal effect present in the behaviour of shellac acids with alkali, as was observed by Wolff (*Farben Z.*, 1922, 27, 3130), that cannot mask the dibasic nature of the pure resin acids, as the forgoing evidences show.

From the known acid value of pure lac resin (58.4), its equivalent weight is 959. Hence, the molecular weight is about 1900, considering it as dibasic. The acid value of pure lac resin generally varies between 55 and 60. Molecular weights, therefore, would be between 1860 and 2000 from the above considerations. From similar considerations soft resin appears to be mono-basic. The acid value of soft resin is generally more than 100 and lies within the limits 100 and 110. Hence, its molecular weight is between 510 and 560, considering it to be mono basic. These values calculated from the consideration of basicity of shellac acids are in good agreement with those previously obtained by the Rast method (*cf. Annual Rep. Indian Lac Res. Inst.*, 1937-38, p. 3), as will be evident from the following table. It should be remembered, however, that as shellac contains both soft and pure resins in the proportion of about 30% and 70%

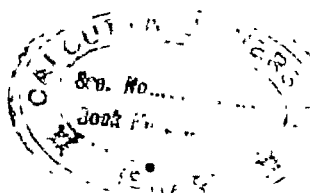
respectively, its average molecular weight is naturally expected to be lower than that of pure lac resin.

Serial No.	Substance.	Acid value.	Molecular weight from A. V. and by Rast method basicity.	
1	Pure lac resin (ether extracted).	58.4	1918	1900-2000
2	Pure lac resin (extracted with ether, dissolved in alcohol, pptd. with water and dried in vacuum)	58.0	1932	1900-2000
3	Soft resin (ether extracted).	101.8	535	513-556

The authors acknowledge their gratitude to Dr. H. K. Sen for his kind interest in the work and to Mr. W. F. Dines of Angelo Bros. Ltd., Calcutta, for his valuable criticisms.

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Received March 20, 1939.



ADSORPTIVE PROPERTIES OF SYNTHETIC RESINS. PART III.

BY S. S. BHATNAGAR, A. N. KAPUR AND MAHENDRA SARUP BHATNAGAR.

The adsorption of a homologous series of mono- and dibasic aliphatic acids by acid and alkali-condensed phenolic resins and by an amino-resin has been studied. In acid-condensed phenolic resins, the adsorption in a homologous series increases with increase in molecular weight, while in alkali-catalysed phenolic resins and amino-resins the order of adsorption is reversed. The adsorption of substituted acetic acids by an amino-resin has also been determined and the influence of the various substituents on adsorption studied. The introduction of acidic groups increases adsorption, while that of basic groups decreases it. The effect of the amino group is much more pronounced than that of hydroxyl group.

In a previous paper from this laboratory Bhatnagar, Kapur and Puri (*J. Indian Chem. Soc.*, 1936, **13**, 679) carried out a quantitative study of the adsorption of non- or weakly dissociated substances by acid-catalysed phenolic resins. They found that the removal of dissolved substances from solution by the resins followed the ordinary laws of adsorption and that in an acid-condensed phenolic resin, the adsorption of organic acids in a homologous series increased strongly and regularly with the increase of molecular weight. Tsuruta (*J. Soc. Chem. Ind. Japan*, 1938, **41**, 129B) studied the sorption of homologous series of fatty acids on an ammonia-condensed phenolic resin and though he obtained results similar to those of Bhatnagar, Kapur and Puri (*loc. cit.*), he found that the amount of sorption decreased with increase in molecular weight.

This anomaly was ascribed by him to the probable presence of traces of ammonia in the adsorbed state in the resin. Though the presence of traces of impurities may lead to increased or diminished general adsorption, it did not appear probable that they could have been responsible for the reversal of the order of adsorption. It appeared more likely that the reversal of the Traube's rule resulted from the differences in the internal structure of the resin. Such reversals are known to occur in the case of activated carbon. Dubinin (*Z. physikal. Chem.*, 1929, **140A**, 81; 1930, **150A**, 145) has shown that the less highly activated charcoals possess very fine ultrapores, while the more highly activated charcoals possess larger pores. The finer pores in the former allow the smaller sorbate molecules to penetrate, but are too small for the larger sorbate molecules and thus bring about a reversal of the order,

In view of the above, it was considered necessary to repeat our own as well as Tsuruta's results. Further, the adsorptive properties of basic resins have been studied at length with respect to a large number of substituted aliphatic acids with a view to throw more light on the mechanism of adsorption in synthetic resins.

EXPERIMENTAL.

Preparation of the Resins.

(i) *Acid-condensed Resin.*—The resin was prepared in the manner described in the previous paper.

(ii) *Ammonia-condensed Resin.*—Equal parts by weight of phenol and formalin (40%) were agitated for an hour in a separating funnel in presence of 5% on the weight of phenol of strong ammonia. A viscous, oily layer of the resin was formed. It was drawn off, washed with water till the filtrate was free from traces of ammonia. The resin, which was in the liquid state, was heated at a temperature ranging from 98° to 100° till it became solid. The solid was finely powdered and passed through a 60 mesh sieve. A large sample of the resin was prepared in the above manner and used throughout all studies.

(iii) *Basic Resins.*—The resin was prepared by dissolving 1 part of *m*-phenylenediamine in 10 parts of dilute hydrochloric acid (1:1). To the clear solution 5 parts of formalin (40%) were added. A dark brown resin was precipitated in about five minutes. The resin was filtered, heated at 100° in presence of excess of hydrochloric acid to render it insoluble in water, and washed with hot water to remove all impurities. The resin was dried, finely powdered and passed through a 60 mesh sieve.

Adsorption Experiments.

The adsorption experiments were carried out in stoppered glass bottles which were thoroughly cleaned, steamed and dried. The powdered resin (1 g.) was weighed out in each bottle and 100 c. c. of the aqueous solutions of the acids were run in. The bottles were vigorously shaken and set aside for 36 hours to attain equilibrium. The supernatant solution (10 c. c.) was pipetted out and run into excess of standard sodium hydroxide solution. The equilibrium concentration (C_e , in millimoles per litre) was determined by titrating the excess of caustic soda against standard hydrochloric acid. Blank experiments were run side by side which gave the original concentration (C_0 , in millimoles per litre). The difference ($C_0 - C_e$) gave the amount adsorbed (x , in millimoles per g. of the resin) from 100 c. c. of the aqueous solutions.

Adsorption in a Homologous Series of Aliphatic Acids.

The results for the adsorption of formic, acetic and butyric acids by acid- and ammonia-catalysed phenol-formaldehyde resins are given in Tables I and II respectively.

TABLE I.

Fatty acids.

Sorption of acids by 1 g. of phenol-formaldehyde resin (acid-catalysed)
from 100 c.c. of aqueous solution.

Acid.	C_0	C_e	x .	% Adsorption.
Formic	10'000	9'800	0.200	2'0
	9'040	8'850	0.190	2.1
	8'030	7.850	0.180	2'2
	5'000	4'860	0.140	2'8
Acetic	10'000	9'500	0.500	5'0
	9'058	8'600	0.458	5'55
	8'040	7'600	0.440	5'47
	7'206	6'800	0.406	5'7
Butyric	10'000	9'100	0.900	9'0
	8'950	8'10	0.850	9'5
	8'000	7.20	0.800	10'0
	7'050	6.30	0.750	10'6

TABLE II.

Fatty acids.

Sorption of acids by 1 g. of phenol-formaldehyde resin (ammonia-catalysed)
from 100 c.c. of their aqueous solution.

Acid.	C_0	C_e .	x .	% Adsorption.
Formic	10'000	7'300	2'700	27'00
	9'100	6'530	2'570	28'23
	8'000	5'650	2'350	29'28
	7'000	4'800	2'200	31'42
Acetic	10'000	7'700	2'300	23'00
	9'100	6'900	2'200	24'17
	7'900	5'900	2.000	25'31
	7'000	5'150	1'850	26'42
Butyric	10.000	8'600	1'400	14'00
	8'900	7'600	1'300	14'61
	8'360	7.100	1'260	15'07
	7'030	6'100	1'150	15'80

It is clear from Table I that in acid-catalysed resin, the adsorption in a homologous series increases with increase in the molecular weight which is in accord with the previous observation of Bhatnagar, Kapur and Pur (*loc.cit.*). Table II shows that, as already reported by Tsuruta, the order of adsorption is reversed in ammonia-catalysed resins.

TABLE III.

Sorption by 1 g. of resorcinol-formaldehyde resin from 100 c.c. of the solution.

	Acid-catalysed resin.				Ammonia-catalysed resin.			
	C ₀ .	C _e .	α .	% Adsorption.	C ₀ .	C _e .	α .	% Adsorption.
Formic	10.00	9.70	0.3	3.3	10.0	6.3	3.7	37.0
Acetic	10.00	9.30	0.7	7.0	10.0	7.0	3.0	30.0
Butyric	10.00	8.70	1.3	13.0	10.0	8.0	2.0	20.0

From Table III we again observe that the normal order of adsorption holds in the case of acid- and reverse order in alkali-catalysed resorcinol-formaldehyde resins. It appeared improbable that the adsorbed traces of ammonia or other impurities could be responsible, as suggested by Tsuruta, for the reversal of the order of adsorption. The ammonia-condensed resin was, therefore, refluxed with hydrochloric acid for two hours with constant stirring to ensure complete removal of adsorbed ammonia. The resin after having been washed free of hydrochloric acid, dried, powdered and sieved still showed the same order of adsorption with the difference that there was a general decrease in the amounts adsorbed. This experiment clearly showed that the reversal of the order of adsorption could not have been due to the presence of traces of ammonia as impurity. It is more likely that the reversal is due to the difference in the internal structure of the acid- and ammonia-condensed resins. It is well known that ammonia and alkalis in general are more powerful condensing agents than hydrochloric acid. The alkali-condensed resins are, therefore, more highly polymerised and possess very fine ultrapores, while acid-condensed resins possess larger pores. This would explain the reversal of the order of adsorption, since the smaller sorbate molecules can penetrate the ultrapores, which are too small for the larger sorbate molecules. Similar explanation for the observance of Traube's rule and its reversal in various activated charcoals was given by Dubinin. It will

be seen from Tables I, II and III that the ammonia condensed resins invariably show much greater adsorption than the acid-condensed resins.

TABLE IV.

Monobasic acids.

Sorption of acids by 1 g. of *m*-phenylenediamine resin from 100 c.c. of their aqueous solutions.

Acids.	C ₀ .	C _e .	α .	% Adsorption.
Formic	10.050	5.981	4.069	40.37
	9.346	5.320	4.026	43.00
	8.223	4.299	3.924	47.73
	7.476	3.645	3.831	51.25
Acetic	10.090	6.354	3.736	37.04
	9.030	5.467	3.563	38.45
	8.037	4.673	3.364	41.86
	7.010	3.832	3.178	45.33
Butyric	10.000	7.196	2.804	28.04
	8.738	6.120	2.618	30.00
	7.943	5.467	2.476	31.17
	7.010	4.673	2.337	33.33

TABLE V.

Dibasic acids.

Sorption of acids by 1 g. of *m*-phenylenediamine resin from 100 c.c. of their aqueous solutions.

Acids.	C ₀ .	C _e .	α .	% Adsorption.
Oxalic	9.9060	1.1215	8.7845	88.68
	8.9730	0.9346	8.0384	89.58
	7.8500	0.7476	7.1024	90.48
	7.0100	0.6074	6.4026	91.33
Malonic	10.090	1.402	8.688	86.11
	8.925	1.028	7.897	88.48
	8.409	0.8879	7.5211	89.44
	7.523	0.6541	6.8689	91.30
Succinic	11.400	2.990	8.410	73.77
	9.438	2.336	7.102	75.25
	8.223	1.865	6.358	77.27
	7.290	1.588	5.702	78.20
Adipic	10.230	3.083	7.147	70.14
	9.159	2.523	6.636	72.45
	8.178	2.055	6.123	74.86
	7.296	1.682	5.608	76.92

Similarly if we consider the adsorption of acids by basic resins. (Tables IV and V), we find that the amount of adsorption is much greater than either in acid- or in alkali-catalysed phenolic resins. Again we notice that the order of adsorption is the same as in the case of alkali-catalysed phenolic resins *i.e.* inverse to that of the normal Traube rule. It appears that the adsorption of acids by synthetic resins is governed by two factors (a) surface forces and (b) forces resulting from physicochemical action of hydrogen ions with basic groups. The adsorption of fatty acids by acid-condensed phenolic resins is a physical phenomenon and the order of adsorption can be qualitatively accounted for on the Gibbs-Thompson law.

TABLE VI.

Influence of ionisation on adsorption.

Acids	% Adsorption from 0.01M solutions.		Ionisation constant at 25°C.
	Phenolic resin (NH ₃ condensed).	<i>m</i> -Phenylene- diamine resin.	
Formic	27.00	40.47	2.14×10^{-4}
Acetic	23.00	37.04	1.86×10^{-5}
Butyric	14.00	28.04	1.48×10^{-5}
Oxalic	60.0	88.68	3.8×10^{-2}
Malonic	46.5	86.11	1.61×10^{-3}
Succinic	38.0	73.77	6.6×10^{-5}
Adipic	21.0	70.14	3.7×10^{-5}

In the case of alkali-condensed phenolic and amino-resins, both physical and chemical forces came into play, the latter masking the effect of the former; the absorbate molecules penetrate the capillary structure of the resin depending on the interfacial tension relationships and their accommodation in the capillary structure of the resin. Further, from Table VI it will be seen that the adsorption in a homologous series is greater, the greater the ionisation constant of the acid. This clearly shows that in the case of amino or basic resins, the adsorption is primarily chemical depending on the interaction of the hydrogen ions with the basic groups in the resin, the stronger acids showing greater adsorption than comparatively weak ones.

TABLE VII.

Influence of substituents on adsorption

Sorption of substituted acetic acids by 1 g. of *m*-phenylenediamine resin from 100 c. c. of their aqueous solutions

Acids.	C_0	C_e	x	% Adsorption
Acetic	10.090	6.354	3.736	37.04
	9.030	5.467	3.563	39.45
	8.037	4.673	3.364	41.86
	7.010	3.832	3.178	45.33
Chloracetic	10.50	4.10	6.40	60.95
	9.60	3.60	6.00	62.50
	8.35	2.90	5.45	65.27
	7.35	2.40	4.95	67.35
Dichloracetic	10.50	2.40	8.10	77.14
	9.40	2.00	7.40	78.72
	8.40	1.60	6.80	79.00
	7.30	1.35	5.95	81.50
Trichloracetic	10.00	1.60	8.40	84.00
	9.00	1.40	7.60	84.44
	8.10	1.20	6.90	85.18
	7.05	1.00	6.05	85.81
Phenylacetic	10.00	2.70	7.30	73.00
	9.00	2.30	6.70	74.44
	8.30	2.00	6.30	76.00
	7.00	1.50	5.50	78.57
Cyanacetic	10.40	3.50	6.90	66.34
	9.50	3.00	6.50	68.42
	8.30	2.40	5.90	71.08
	7.30	1.90	5.40	73.97
Aminoacetic	12.00	11.00	1.00	8.33
	7.00	6.25	0.75	10.70
	5.35	4.70	0.65	12.10
	3.90	3.35	0.55	14.10
Hydroxyacetic	10.4	7.2	3.20	30.8
	9.2	6.1	3.10	33.7
	8.1	5.1	3.00	37.3
	7.1	4.2	2.90	40.8

The adsorption of acetic, mono-, di- and tri-, phenyl-, cyano-, amino- and hydroxyacetic acids by *m*-phenylenediamine resin has been determined and the results are recorded in Table VII. It will be seen from the table that the introduction of acidic groups increases adsorption, while that of basic groups decreases it. Further, the effect of the amino group in lowering the adsorption is much more pronounced than that of the hydroxyl group, due probably to the former being more basic than the latter. Similar results were also obtained by Bartell and Miller (*J. Amer. Chem. Soc.*, 1893, **15**, 1106) who showed that the introduction of the hydroxyl or amino group in an organic acid decreased adsorption on carbon to an extent depending on the nature of the acid.

TABLE VIII.

Influence of ionisation on adsorption.

Acids.	% Adsorption from 0.01M soln.	Ion const. at 25°C.	Acids	% Adsorption from 0.01M soln.	Ion. const. at 25°C.
Acetic	37.04	1.85×10^{-5}	Cyanacetic (nitrilomalonic acid)	66.34	3.56×10^{-3}
Chloroacetic	60.95	1.52×10^{-3}	Hydroxyacetic (glycollic acid)	30.80	1.51×10^{-4}
Dichloroacetic	77.14	5.00×10^{-2}	Aminoacetic (glycocoll)	8.33	3.4×10^{-10}
Trichloroacetic	84.00	2.00×10^{-1}			
Phenylacetic	73.00	5.45×10^{-5}			

In Table VIII are given the percentage adsorptions of the substituted acetic acids and their ionisation constants at 25°. It will be seen that here again the general relation between adsorbability and ionisation holds except in the case of hydroxyacetic acid. The influence of the hydroxyl group in depressing the adsorption is well known and in this case appears to be potent enough to overcome the increased adsorption due to a higher dissociation constant.

A STUDY OF THE PERIODATES OF COPPER.

By R. K. BAHL AND SURJIT SINGH.

Quaternary cupric paraperiodate was formed by the interaction between disodium paraperiodate ($\text{Na}_2\text{H}_3\text{IO}_6$) and copper sulphate, and between paraperiodic acid and copper carbonate. Heptahydrated cupric paraperiodate, was formed by the action of paraperiodic acid on copper acetate. On dehydrating this salt the pentahydrated cupric paraperiodate was formed. The vapour pressures of the hepta- and pentahydrates were found to be 5 mm. and 3 mm. respectively. The trihydrate could not be obtained by further dehydration.

It has been stated (Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry", 1922, II, p. 412) that the interaction of copper salts with alkali periodates or paraperiodic acid results in the formation of five periodates of copper. There are, however, many inconsistencies in the results of the previous workers. For example, Rammelsberg (*Ann. Physik*, 1868, **134**, 519) obtained a green powder by treating copper carbonate with paraperiodic acid having the composition 5CuO , I_2O_7 , $5\text{H}_2\text{O}$, cupric paraperiodate pentahydrate $\text{Cu}_5(\text{IO}_6)_2 \cdot 5\text{H}_2\text{O}$, whereas Langlois (*Ann. chim. phys.*, 1836, **34**, 257) got a bright green compound, quaternary cupric paraperiodate, Cu_2HIO_6 or $\text{Cu}_4\text{I}_2\text{O}_{11}$, H_2O by the same treatment.

The results obtained by us confirm Langlois observations. We have been able to prepare only two salts. The quaternary cupric paraperiodate Cu_2HIO_6 or $\text{Cu}_4\text{I}_2\text{O}_{11}$, H_2O and the heptahydrated cupric paraperiodate $\text{Cu}_5(\text{IO}_6)_2 \cdot 7\text{H}_2\text{O}$.

A study of the periodates of copper involved:

- (a) The preparation of an alkali periodate and paraperiodic acid, and
- (b) Methods of analysing copper periodates.

(a) Disodium paraperiodate $\text{Na}_2\text{H}_3\text{IO}_6$ and paraperiodic acid H_5IO_6 or HIO_4 , $2\text{H}_2\text{O}$ were prepared by Wells' method (*Amer. Chem. J.*, 1901, **26**, 278) as modified by Partington and Bahl (*J. Chem. Soc.*, 1934, 1086). The acid was obtained in the form of transparent, colourless, prismatic crystals, confirming the observations of the latter workers. The melting point of three different specimens prepared at different times was found to be 122° .

(b) Analysis of the periodates of copper :—

(i) The copper was estimated as cupric oxide, by heating a weighed amount of the salt to a constant weight.

(ii) The oxygen in the sample was estimated by the method adopted by Partington and Bahl (*loc. cit.*).

(iii) The iodine was estimated by Kimmin's method as modified by Partington and Bahl (*loc. cit.*). The amount of iodine liberated by the copper present in the salt, was taken into account.

EXPERIMENTAL.

Quaternary Cupric Paraperiodate, Cu_2HIO_6 or $\text{Cu}_4\text{I}_2\text{O}_{11}$, H_2O .

Preparation.—(A) The fine bluish green suspension of copper carbonate in distilled water was treated with a solution of paraperiodic acid, drop by drop, with continuous stirring. The slow evolution of carbon dioxide at the room temperature became brisk on warming. As the reaction proceeded, the sky-blue precipitate changed first to dark green and finally to yellowish green.

(B) A suspension of disodium paraperiodate $\text{Na}_2\text{H}_3\text{IO}_6$ (about 3 g. in 20 c.c. of water) was boiled with an excess of copper sulphate solution, that being ensured by the bluish colour of the supernatant liquid, when the precipitate was immediately formed.

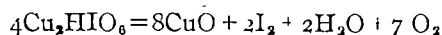
The precipitates obtained in the above two methods were filtered, washed and dried at 45° in an electric air oven. The dry salt was a yellowish green powder and was crystalline under the microscope.

The compound was analysed for copper, iodine and oxygen (Table I)

TABLE I.

Sample.	Copper.	Iodine.	Available oxygen.
(A) 1	35.80%	37.10%	15.50%
2	35.98	36.20	15.72
3	35.88	36.51	15.70
(B) 1	35.89	37.40	15.25
2	36.10	37.51	15.26
3	35.95	37.08	15.64

The calculated values of copper and iodine are 36.20 and 36.17% respectively. The available oxygen according to the decomposition



is 15.95%.

We were unable to obtain cupric paraperiodate pentahydrate, $\text{Cu}_5(\text{IO}_6)_2 \cdot 5\text{H}_2\text{O}$ by the method (A) as claimed by Rammelsberg (*loc. cit.*),

Heptahydrated Cupric Paraperiodate $\text{Cu}_5(\text{IO}_6)_2, 7\text{H}_2\text{O}$.

A dilute solution of paraperiodic acid was added, drop by drop, to a dilute solution of copper acetate. A bright green precipitate thus formed, was filtered, washed and dried at 45° in an electric air oven. The deep green dry salt was analysed.

TABLE II.

Sample.	Copper.	Iodine.	Available oxygen.
1	34.44%	29.10%	12.65%
2	35.43	29.12	12.20
3	34.65	26.5	12.12
4	35.68	29.0	12.62

The calculated values of copper and iodine are 35.83 and 28.54% respectively. The available oxygen according to the decomposition

$2\text{Cu}_5(\text{IO}_6)_2, 7\text{H}_2\text{O} = 10\text{CuO} + 2\text{I}_2 + 14\text{H}_2\text{O} + 7\text{O}_2$
is 12.58%.

These results prove definitely that the compound obtained by the above reaction is heptahydrated cupric paraperiodate $\text{Cu}_5(\text{IO}_6)_2, 7\text{H}_2\text{O}$ and not the sky-blue cupric metaperiodate, $\text{Cu}(\text{IO}_4)_2$, as described in the literature (Mellor, *loc. cit.*); the calculated values for the latter being Cu, 14.6% ; I, 54.76% ; O(available), 25.14%

Effect of Heat on Periodates of Copper.

1. No loss of weight was found by heating quaternary copper paraperiodate, Cu_2HIO_6 or $\text{Cu}_4\text{I}_2\text{O}_{11}, \text{H}_2\text{O}$, up to 120° in an electric air oven or upto 110° in vacuum.

2. Heptahydrated copper paraperiodate $\text{Cu}_5(\text{IO}_6)_2, 7\text{H}_2\text{O}$ was taken in an uncovered weighing bottle and heated in an air oven. There was no loss of weight upto 70° . At 74° the dehydration took place yielding pentahydrated copper paraperiodate $\text{Cu}_5(\text{IO}_6)_2, 5\text{H}_2\text{O}$. The dehydration at this temperature was slow and took five hours. There was no further loss upto 120° .

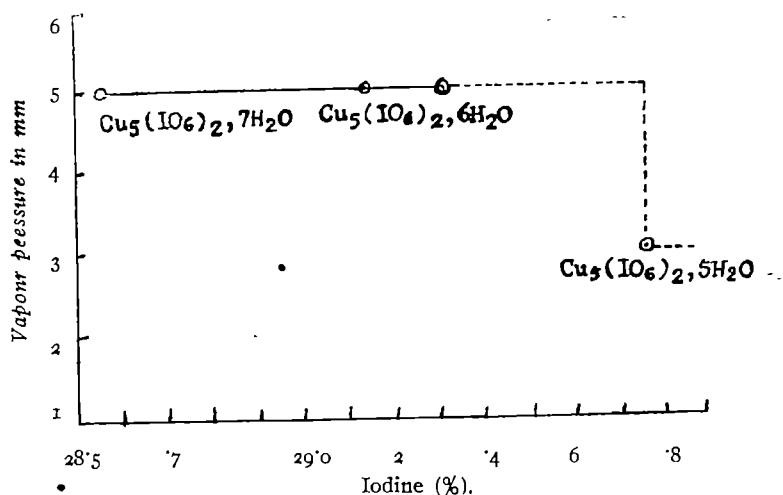
The same salt when heated in a vacuum desiccator dehydrated at a lower temperature 65° , and took 4 hours for completion. There was no further loss upto 100° . The analysis of the pentahydrate is as shown in Table III.

TABLE III.

	1st sample.	2nd sample.	Calculated.
Available oxygen	13.3%	13.13%	13.11%
Copper	37.4	37.32	37.21
Iodine	29.3	29.45	29.75

A study of the vapour pressure of these hydrates confirmed the existence of the pentahydrated copper paraperiodate $\text{Cu}_5(\text{IO}_6)_2, 5\text{H}_2\text{O}$.

Vapour pressure of copper salt at 74°.



In these experiments the salt was placed in a small tube, connected with a vacuum pump and a manometer which recorded a high vacuum at the room temperature. The stop-cock connecting the pump was then closed, so that only the tube and the manometer were left connected together. The vapour pressure of the heptahydrate was found to be 5 mm. and that of the pentahydrate to be 3 mm. A mixture of the two hydrates always indicated a vapour pressure of 5 mm. at 74°.

The graph shows the vapour pressures of the two hydrates. The points shown in the graph were obtained by dehydrating the heptahydrate at 74°.

THE ESTIMATION OF ANEURIN BY THIOCHROME REACTION WITH PULFRICH PHOTOMETER.

BY A. MUKHERJI.

A modification of Jansen's method for the estimation of aneurin in different substances by converting the aneurin to thiochrome is described. It consists in carrying out the oxidation of aneurin in an atmosphere of carbon dioxide and later on in estimating the thiochrome by means of analysis with quartz lamp and Pulfrich's photometer. The results agree with the findings of other workers using Cohen fluorometer.

The fact that aneurin is oxidised to thiochrome (Peters, *Nature*, 1935, 135, 107) in an alkaline medium (Barger *et al*, *Ber.*, 1935, 68, 2257) has been utilised by Jansen (*Rec trav. chim.*, 1936, 55, 1046) for quantitative determination of aneurin. It has later on been modified by Westenbrink and Goudsmit (*Rec. trav. chim.*, 1937, 56, 803) for measuring the same in urine, but undoubtedly the same can be extended for measuring aneurin in other substances, normally liquid. Pyke has used the same method for determining aneurin in solid substances where he simply powders the substance and dissolves in water containing 1% hydrochloric acid and uses the liquid recovered from the same (*Biochem. J.*, 1937, 31, 1958). Karrer and Kubli (1937, 20, 369) used the method of Jansen and by some modification changed the fluorescing colour from rich blue to violet and also the intensity of the fluorescence was increased considerably which enabled them to measure the intensity with naked eye with another standard. Jansen oxidised the aneurin in an atmosphere of nitrogen gas and measured the fluorescence of the resulting thiochrome by means of a Cohen fluorometer, in which the fluorescence is converted into electric current by means of a photoelectric cell and led off to a ballistic galvanometer, in the mirror of which a beam of light was incident. The movement of the light is recorded over a graduated scale. In the present communication a method is described by which the fluorescence itself is being measured by means of a Pulfrich photometer using quartz analysis lamp as the source of light. The nitrogen gas is replaced by carbon dioxide which gives stability to the thiochrome formed and is also easily procurable. The amount of isobutanol required is much less and so adds to the economy where large number of estimations are to be made.

E X P E R I M E N T A L.

For each estimation 60 and 75 c. c. of the liquid to be examined were taken and diluted with water to 240 and 300 c. c. respectively. It was brought to a p_H of about 3 by adding hydrochloric acid. Ten centrifuge tubes were taken and four of them received 30 c. c. of the solution in each tube. The remaining 180 c. c. were divided in two parts and mixed with different amounts of aneurin, usually between 10-50 γ , 30 c. c. of the solution were then put in each of the six tubes. Fuller's earth was added in 100 mg. parts to each tube, stirred for about 3-5 minutes and centrifuged. The supernatant liquid was thrown off and Fuller's earth was washed first with distilled water which was made acid with hydrochloric acid to a p_H of about 3, and then with absolute alcohol or rectified spirit. The earth was then dried in an oven at a temperature of about 80-100°. The aneurin, which was adsorbed on the Fuller's earth, was then eluted by 2 c. c. of methyl alcohol while a stream of pure carbon dioxide was passed through the mixture from a carbon dioxide cylinder. After about two minutes 1 c. c. of 30% caustic potash solution was added and at intervals of half a minute 1% potassium ferricyanide, 2 c. c. of water, and 3 c. c. of isobutyl alcohol were added and finally after 1 minute the tube was corked and centrifuged for a short time, till the isobutyl alcohol layer was clear. It is to be emphasised here that till before corking carbon dioxide was continuously passed through the solution. Amount of potassium ferricyanide required for formation of maximum amount of thiochrome is variable and depends both on the amount of aneurin and the nature of foreign substances present in the test solution and therefore different amounts were added in different tubes. It has been found by Jansen *et al* (*loc. cit.*) that the amount of 1% potassium ferricyanide required lies within the limit of 0.6-1 c. c. So each set of tubes got 0.6, 0.8 and 1 c. c. of potassium ferricyanide, except that in the first set of four solutions without added aneurin, the fourth one had no potassium ferricyanide and served as a control. The estimation of the thiochrome and also of the aneurin was then carried out by means of Pulfrich photometer and analysis quartz lamp. The bulb of the lamp was brought to a level at right angles with the cell-holder of the photometer.

A filter, filled with 10% copper sulphate solution, was used with the mercury. As it is very important to have equal illumination, light from that portion of the bulb was used only which was seen glowing equally bright

through the filter, and could be conveniently marked off with a marking pencil over the metal frame of the filter. As with fluorometry it is not possible to have the so-called absolute measurements of colorimetry, it is essential to have another fluorescing solution whose spectral energy would approach the test solution as much as possible and at the same time be easily available. With the photometer a piece of uranium glass was provided which was more green fluorescing than blue, while the fluorescence of the thiochrome was rich blue and so could not be utilised for the purpose. Quinine sulphate in acid solution fluoresces both green and rich blue, and is easily available and quite stable at least for several months as far as fluorescence is concerned. For most of the cases it will be sufficient to use 1 mg % of the quinine sulphate solution bringing the p_H of the solution to 3 with dilute hydrochloric acid. It has been found that greater fluorescence is obtained with thinner cells. Cells, 2.47-2.50 mm. thick, have been found to be quite suitable in fluorometry. The two cells filled with the quinine sulphate solution were placed in the cell-holder till equal illumination was obtained for both. One of the cells was cleaned and filled with the test solution containing the formed thiochrome and placed in the cell-holder, while the other cell containing quinine solution was lying in the other cell-holder. The drum on that side, on which the solution fluoresces, was kept fully open, while the drum on the other side was adjusted till the intensities of light were as much as possible equal on both sides of the field of illumination. The readings were then noted in terms of the percentage of light. This is a case of real fluorescence and so no question of extinction of light arises and we do not require to note the extinction coefficient which is found in red letters by the side of the scale noting light transmitted in percentage. It will further be found from the following data that the reading is exactly proportional to the concentration of thiochrome and so of aneurin contained in the test solution. In the case of solutions containing much suspension it must be centrifuged off before proceeding with the estimation, for otherwise they will be precipitated with the Fuller's earth and when finally dried may form a hard mass very difficult to be suspended in methanol during elution of the vitamin. The foreign substances also, when present in large quantities, interfere with the more precise determinations. In the case of solid substances they are first powdered and soaked in water, brought to p_H 3 by keeping with hydrochloric acid for about 24 hours, shaking now and then or preferably stirring continually by means of an electric stirrer. Afterwards it was filtered and the filtrate used for estimation of aneurin. Where the substance is supposed to contain very little aneurin it is

better to take at least 100 g. of the substance and dissolve it in 400 c.c. of the acidulated water. The following are the results of some of the determinations in various substances.

With pure aneurin solutions containing 10, 20, 25, 27 and 50 γ , keeping the drum fully open on this side, i.e., at 100, the quinine values were 5.9; 5, 13, 18 and 25.5 which are nearly proportional to aneurin contents.

S A M P L E I.

Urine diluted to four times its volume.

Quinine value	Urine value	1% Pot. ferricvanide..	
3.37	100	0 c.c.	
3.5	100	0.8	
4.0	100	1.0	
	Urine value + 97B ₁ .		
4.0	100	0.6	
4.25	100	0.8	
5.2	100	1.0	
	Urine value + 277B ₁ .		
5.0	100	0.6	
5.5	100	0.8	
7.5	100	1.0	
Blank	Urine.	Urine + 97B ₁ .	Urine + 277B ₁ .
(without Pot. ferricvanide)			
3.37	4	5.2	7.5
Urine (blank)	= 0.63 = A		
Urine + 97B ₁ -urine (blank)	= 1.2 = B		
Urine + 277B ₁ -urine (blank)	= 3.5 = C		

It will be seen that C is about three times B corresponding with the amount of aneurin added. Now A represents aneurin contained in 100 c.c. of the dilute urine, therefore in the original urine the aneurin would be represented by 0.63×4 , i.e., 2.52% of the fluorescence of the quinine. The percentage of fluorescence of quinine represented by 1.2 and 3.5 represents respectively the fluorescence of thiochrome formed from 9 and 27 γ of

aneurin. Hence the urine contained 197 aneurin per 100 c.c. It is important to note that the person (a vegetarian) used to take little water and the urine was scanty and concentrated.

S A M P L E II.

Urine diluted to four times its volume.

Quinine value.	Urine value.	1% Pot. ferricyanide
2.75	100	0 c.c.
3.37	100	0.6
3.16	100	0.8
3.3	100	1.9
	Urine value + 87B ₁ .	
4.125	100	0.6
4.125	100	0.8
4.75	100	1.0
	Urine value + 187B ₁ .	
5.5	100	0.6
6	100	0.8
7.25	100	1.0
Urine + 87 aneurin - urine = 1.38 = A		
Urine + 187 aneurin - urine = 3.38 = B		
Urine (blank) = 0.62 = C		

It is found that B is about two and half times A corresponding to the aneurin added. The figures 1.38 and 3.38 represent respectively 8 and 18 aneurin so that 0.62×4 or 2.48 represents about 147 aneurin and is contained in 100 c.c. of original urine.

S A M P L E III.

Urine was diluted to four times its volume. From now only one reading will be given for each series corresponding with the maximum fluorescence obtained with the optimal amount of potassium ferricyanide.

Quinine value	Urine value.	1% Pot. ferricyanide.
3.37	100	0.8 c.c.
2.7	100	
	Urine value + 197B ₁ .	
8.0	100	
	Urine value + 387B ₁ .	
13.25	100	

Urine (blank) = 0.67 = A

Urine + 197B₁ - urine = 4.63 = B

Urine + 387B₁ - urine = 9.88 = C

The figures in C are about twice those in B corresponding to the aneurin content added so that 197B₁ is represented by 4.63 or the aneurin contained in the original urine is about 10.57 aneurin per 100 c.c.

S A M P L E IV.

Urine diluted to four times.

Quinine value.	Urine value.	1% Pot. ferricyanide.
2.6	100	0 c.c.
3.12	100	0.8
	Urine value + 197B ₁ .	
6.5	100	1
	Urine value + 387B ₁ .	
9.0	100	1
Blank	Urine + 197B ₁ .	Urine + 387B ₁ .
2.6	3.12 6.5	9

Urine (blank) = 0.52 = A

Urine + 197B₁ - urine = 3.38 = B

Urine + 387B₁ - urine = 6.38 = C

Readings in C are about twice those in B corresponding to the aneurin added. As per calculation above the aneurin per 100 c.c. of the urine is 11.57.

Polished rice.

100 G. of the rice powder soaked in 600 c.c. of water (p_H 3) for 24 hours with shaking at intervals, afterwards filtered and used for aneurin estimation.

Quinine value.	Rice soln	1% Pot ferricyanide.
2.87	100	0 c.c.
3.5	100	1
	Rice soln. + 207B ₁ .	
6.5	100	1
	Rice soln. + 417B ₁	1
8.25	100	1

Therefore 100 g. of the sample of rice contain about 337 aneurin or 16.5 international units.

Carrot.

100 G. of the minced substance were soaked in 400 c.c. of water (p_H 3) for 24 hours with shaking at intervals, filtered and the filtrate used for estimation of aneurin.

Quinine value	Carrot soln.	1% Pot. ferricyanide.
3.6	100	0 c.c.
5	100	0.8
	Carrot soln. + 157B ₁ .	
7.2	100	0.8
	Carrot soln. + 307B ₁	
9.7	100	0.8

Carrot soln. (blank) = 1.4 = A

Carrot soln. + 157B₁ - carrot (blank) = 2.2 = B

Carrot soln. + 307B₁ - carrot (blank) = 4.7 = C

Therefore 100 g. of the substance contain about 407 aneurin or 20 I.U

In a similar way aneurin contents of some other substances were determined. A sample of bread was found to have 6.57 aneurin per 100 g. of the substance. A sample powder designated as vitamin B complex by a Calcutta firm contained 2647 aneurin per 100 g. of the substance. This was undoubtedly a sample of aneurin adsorbed on Fuller's earth and as such the estimation was not done in the usual way. 0.5 G. of the substance was put in each of the 10 centrifuge tubes and four of them treated with methanol, KOH solution, etc., from the very beginning. Three of the rest

were treated with 12.5γ aneurin and the other three with 25γ aneurin, the solution was centrifuged off, washed with water at p_H 3, and alcohol and dried at 100°. Then the estimation was done as usual starting with methanol. Two of the three preparations were liquid and estimation was made as usual. One of them contained 34.6γ aneurin per 100 c.c. and the other contained 60γ aneurin per 100 c.c. of the substance.

DISCUSSION.

From the above results we are in a position to say that aneurin can be estimated by the present modification of Jansen's method using a Pulfrich photometer instead of a fluorometer and the reasons are as follows: (1) the readings obtained are very approximately proportional to the aneurin present with pure solutions; (2) with impure solutions the readings obtained after adding different amounts of aneurin subtracted from the reading without addition of aneurin are closely proportional to the amount of aneurin added; (3) the aneurin contents of carrot, polished rice, etc., agree with the biological values; (4) the aneurin values of human urine found here vary from 10.5 to 19γ per 100 c.c. Westenbrink and Goudsmit (*Ext. Arch. Neerl. Physiol.*, 1937, 22, 319 and *Nederl. Tijdschr. Geneesk.*, 1938, 82, 518) found 5-20γ aneurin per 100 c.c. Karrer (*Helv. Chim. Acta*, 1937, 20, 1147) found 10.7-14γ aneurin per 100 c.c. of urine. The advantage with this method is that it does not require a special instrument for the estimation and the use of carbon dioxide instead of nitrogen gives stability to the thiochrome formed which has a distinct advantage in estimating the fluorescence in ultraviolet light which otherwise is destroyed quickly, and besides carbon dioxide is a much more common thing in a laboratory than nitrogen.

Compared with the electrical measurement of the fluorescence there is one disadvantage with the present method and that is the personal factor involved in matching the intensities. But errors that arise in this connection have been greatly reduced by increasing the fluorescence of the test solution simply by decreasing the quantity of isobutanol from 13 c.c. to 3 or 4 c.c. which is also appreciably economical when aneurin contents of large number of samples are to be determined.

INTERACTION OF SULPHURYL CHLORIDE WITH
ARYLAMIDES OF AROMATIC ACIDS. PART II.
ORIENTING INFLUENCES OF GROUPS IN SUB-
STITUTION REACTIONS IN AROMATIC
COMPOUNDS:

BY (LATE) N. W. HIRWE, G. V. JADHAV AND D. R. SUKHTANKAR.

Sulphuryl chloride gives chloro derivatives with arylamides of salicylic acid, having chlorine both in the acidic and the basic parts of the molecule. Varying proportions of sulphuryl chloride show that chlorine enters first in position *para* to OH and *para* to NHCOR, then *ortho* to OH and then *ortho* to NHCOR according to the nature of the reacting amide. With arylamides of *o*-methoxy- and *o*-chlorobenzoic acids and *o*-toluic acid, products with chlorine only in the basic part are obtained. The constitution of compounds is proved by hydrolysis and synthesis.

It is known that the ease with which an incoming group enters a substituted benzene nucleus as well as the position which the new substituent would take, depend upon the nature of the substituent already present and the reagent used for substitution. It has also been generally accepted that the substituents such as OH, NH₂, CH₃ and halogens are mainly *ortho* and *para* directing and the order of their reactivity is OH > NH₂ > halogens > CH₃ and the substituents such as COOH, SO₃H and NO₂ are predominantly *meta* directing, their order of reactivity being NO₂ > SO₃H > COOH.

The present work concerns the reaction of sulphuryl chloride with arylamides of salicylic, *o*-methoxybenzoic, *o*-toluic and *o*-chlorobenzoic acids.

It has been observed in Part I (*J. Indian Chem. Soc.*, 1938, **15**, 649) that chlorine always enters the basic part of the molecule in the case of simple benzoic acid derivatives, but the introduction of the OH group in benzene ring increases the reactivity of the acidic part of the molecule and substitution first takes place in the acidic part of the molecule. It has been observed that even when the reacting substances are in the molecular proportion of 1:1 in the case of salicylanilide and salicyl-*m*-toluidide, two products are obtained, viz.,

(1) 5-Chlorosalicylanilide and (2) 5-chlorosalicyl-4'-chloroanilide and
(1) 5-chlorosalicyl-*m*-toluidide and (2) 5-chlorosalicyl-6'-chloro-*m*-toluidide

(Me=1'), in the ratio of 1:9 and 1:4 respectively, showing thereby that the *para* directing influence of OH is stronger than that of $\text{NH}\cdot\text{COR}$. In the case of salicyl-*o*-toluidide and salicyl-*p*-toluidide, 5-chlorosalicyl-5'-chloro-*o*-toluidide and 5-chlorosalicyl-*p*-toluidide (Me=1') are obtained when the reacting materials are in the molecular ratio of 1:1.

When two molecules of sulphuryl chloride are used for one of the amide, the dichloro compounds as above are obtained except in the case of salicyl-*p*-toluidide when no disubstitution product is formed.

Chlorination of the acidic part of the molecule does not take place in the case of the arylamides of *o*-methoxybenzoic acid, *o*-toluic acid and *o*-chlorobenzoic acid, which indicates that the directing power of NHCOR is stronger than that of OCH_3 , CH_3 or chlorine

In the case of *o*-tolu-*m*-toluidide, however, *o*-tolu-4:6-dichloro-*m*-toluidide (Me=1) is formed even when the reacting substances are in the molecular ratio of 1:1 and substitution products are formed in the case of *o*-chlorobenz-*o*-chloroanilide and *o*-chlorobenz-*p*-chloroanilide.

EXPERIMENTAL.

Sulphuryl chloride was gradually added to the amide suspended in dry benzene (about 40 c.c. for 5 g. of the amide) and the mixture, protected from moisture, was boiled under reflux for about 4-5 hours. After the removal of benzene, the reaction product was washed free from sulphuryl chloride with dry petroleum ether. The substances, generally soluble in benzene, alcohol and acetic acid, crystallised from alcohol in colourless needles. Mixtures were separated by fractional crystallisation from alcohol.

The constitution of substances marked (A) was proved by hydrolysis to known amines and acids and also by synthesis (by heating a mixture of the amine, acid and phosphorus trichloride at 120° for about 1 hour).

Substances marked (B) were identified by hydrolysis to the corresponding amines and acids only. In case of substances marked (D) in addition to (A) and (B), the amines were identified through their acetyl derivatives.

Hydrolysis was carried out by heating the substance in a sealed tube with 10-15% caustic potash solution in 50% alcohol at 170° for about 6 hours.

The compounds are described in Table I.

TABLE I.

Amide	Mol. prop of amide to SO ₂ Cl ₂	Product.	M. p.	Formula.	Found.	Required.	Yield.
Salicylanilide	1 : 1	(i) 5-Chlorosalicylanilide (A & D)	203·4°	C ₁₃ H ₁₀ O ₂ NCl	Cl, 14·3%	Cl, 14·3%	7%
		(ii) 5-Chlorosalicyl-4'-chloroanilide (B & D)	215·16°	C ₁₃ H ₉ O ₂ NCl ₂	Cl, 25·1	Cl, 25·2	83
Salicylanilide	1 : 2	5-Chlorosalicyl-4'-chloroanilide*	215·16°	C ₁₃ H ₈ O ₂ NCl ₃	—	—	80
Salicylanilide	1 : 4	(i) 5-Chlorosalicyl-4'-chloroanilide*	215°	C ₁₃ H ₇ O ₂ NCl ₃	—	—	45
		(ii) 3 : 5-Dichlorosalicyl-2' : 4'- dichloroanilide (B & D)	174·75°	C ₁₃ H ₇ O ₂ NCl ₄	Cl, 40·2 N, 4·2	Cl, 40·5 N, 4·0	13
Salicyl-o-toluidide	1 : 1	5-Chlorosalicyl-5'-chloro-o-tolui- dide (B&D)	171·72°	C ₁₄ H ₁₁ O ₂ NCl ₂	Cl, 23·8	Cl, 23·99	60
Salicyl-o-toluidide	1 : 2	5-Chlorosalicyl-5'-chloro-o-tolui- dide*	171·72°	C ₁₄ H ₁₀ O ₂ NCl ₃	—	—	90
Salicyl-o-toluidide	1 : 4	(i) 3 : 5-Dichlorosalicyl-5'-chloro-o- toluidide (B&D)	197·98°	C ₁₄ H ₉ O ₂ NCl ₃	31·9	32·2	10
		(ii) 5-Chlorosalicyl-5'-chloro-o- toluidide*	170·71°	C ₁₄ H ₁₀ O ₂ NCl ₃	—	—	40
Salicyl-m-toluidide	1 : 1	(i) 5-Chlorosalicyl-m-toluidide (C)	144·45°	C ₁₄ H ₁₁ O ₂ NCl	13·8	13·6	10
		(ii) 5-Chlorosalicyl-6'-chloro-m- toluidide (B)	221·22°	C ₁₄ H ₁₁ O ₂ NCl ₂	24·2	23·99	40
Salicyl-m-toluidide	1 : 2	5-Chlorosalicyl-6'-chloro-m- toluidide*	221·22°	C ₁₄ H ₁₀ O ₂ NCl ₃	—	—	75

TABLE I (contd.).

Amide	Mol. prop. of amide to SO ₂ Cl ₂	Product.	M. p.	Formula.	Analysis Found.	Analysis Req.	Yield
Salicyl- <i>m</i> -toluidide	1 : 4	(i) 3 : 5-Dichlorosalicyl-4' : 6'-dichloro- <i>m</i> -toluidide (B)	214-15°	C ₁₄ H ₉ O ₂ NCI ₄	Cl, 38.7%	Cl, 38.9%	10%
Salicyl- <i>p</i> -toluidide	1 : 1	(ii) 5-Chlorosalicyl-6'-chloro- <i>m</i> -toluidide*	221-22°	C ₁₄ H ₁₁ O ₂ NCI ₂	—	—	50
Salicyl- <i>p</i> -toluidide	1 : 2	5-Chlorosalicyl- <i>p</i> -toluidide (C)	216-17°	C ₁₄ H ₁₃ O ₂ NCI	13.3	13.3	30
Salicyl- <i>p</i> -toluidide	1 : 4	5-Chlorosalicyl- <i>p</i> -toluidide*	216°	C ₁₄ H ₁₃ O ₂ NCI	—	—	60
		(i) 3 : 5-Dichlorosalicyl-3'-chloro- <i>p</i> -toluidide (B & D)	164-65°	C ₁₄ H ₁₀ O ₂ NCI ₃	31.9	32.2	20
<i>o</i> -Methoxybenzanilide	1 : 1	(ii) 5-Chlorosalicyl- <i>p</i> -toluidide*	215-16°	C ₁₄ H ₁₂ O ₂ NCI	—	—	50
<i>o</i> -Methoxybenz- <i>o</i> -anisilide	1 : 1	<i>o</i> -Methoxybenz-4-chloroanilide (B & D)	75-76°	C ₁₄ H ₁₃ O ₂ NCI	13.8	13.6	60
<i>o</i> -Toluanilide	1 : 1	<i>o</i> -Methoxybenz-5-chloro- <i>o</i> -anisilide (B)	135-36°	C ₁₅ H ₁₄ O ₂ NCI	12.4	12.2	60
<i>o</i> -Toluanilide	1 : 2	<i>o</i> -Tolu-4-chloroanilide (A & D)	133-34°	C ₁₄ H ₁₃ ONCI	14.3	14.5	—
		(i) <i>o</i> -Tolu-2 : 4-dichloroanilide (B & D)	128°	C ₁₄ H ₁₁ ONCI ₂	25.1	25.3	70
		(ii) <i>o</i> -Tolu-4-chloroanilide*	132-33°	C ₁₄ H ₁₃ ONCI	—	—	—
<i>o</i> -Tolu- <i>o</i> -toluidide	1 : 1	<i>o</i> -Tolu-5-chloro- <i>o</i> -toluidide (B & D)	182°	C ₁₅ H ₁₄ ONCI	13.4	13.7	70
<i>o</i> -Tolu- <i>m</i> -toluidide	1 : 1	<i>o</i> -Tolu-4 : 6-dichloro- <i>m</i> -toluidide (B & D)	120-21°	C ₁₅ H ₁₃ ONCI ₂	23.9	24.1	—
<i>o</i> -Tolu- <i>p</i> -toluidide	1 : 1	<i>o</i> -Tolu-3-chloro- <i>p</i> -toluidide (B & D)	119-20°	C ₁₅ H ₁₄ ONCI	13.5	13.7	—
<i>o</i> -Chlorobenzanilide	1 : 1	<i>o</i> -Chlorobenz-4-chloroanilide (A & D)	119-20°	C ₁₅ H ₉ ONCI ₂	26.5	26.7	80
<i>o</i> -Chlorobenz- <i>m</i> -chloroanilide	1 : 1	<i>o</i> -Chlorobenz-3 : 4-dichloroanilide (B)	143°	C ₁₅ H ₈ ONCI ₃	35.2	35.4	—

N.B.—(i) In the case of toluidides and anisilide Me and OMe are numbered 1.

(ii) Substances marked * were identified by mixed m.p. with authentic specimens.

The authors thank Dr. T. S. Wheeler for his interest in the work and one of them (G. V. J.) thanks the University of Bombay for a grant.

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Received April 11, 1939.

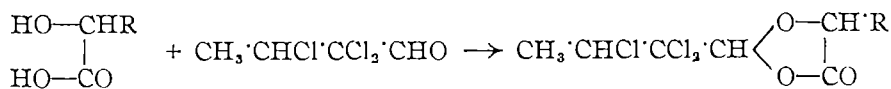
CHLORALIDES. THE CONDENSATION OF BUTYLCHLORAL WITH α -HYDROXYCARBOXYLIC ACIDS.

BY N. M. SHAH.

The condensation of butylchloral hydrate with α -hydroxycarboxylic acids in presence of sulphuric acid has been studied in order to compare its behaviour with that of chloral.

The condensation of chloral with α -hydroxycarboxylic acids has been studied by numerous investigators (*vide* Shah and Alimchandani, *J. Indian Chem. Soc.*, 1934, 11, 545 for details of references), but the corresponding chloralides derived from butylchloral are little known. In the work described here, an attempt to condense butylchloral hydrate with malic, tartaric, citric, mandelic and benzilic acids has been made.

The general method adopted throughout this work was to dissolve the two components in concentrated sulphuric acid and after 24 hours to pour the mixture on to ice.



Malic, tartaric and citric acids give their corresponding butylchloralides. The behaviour of citric and malic acids in this condensation is similar to that with chloral. Tartaric acid on condensation gives only a small quantity of its butylchloralide, most of the butylchloral hydrate being recovered unchanged. This is in contrast to the behaviour of chloral with this acid. Attempts to get the mono-condensation product are unsuccessful. It may be mentioned here that the yield of the butylchloralides is uniformly inferior compared to the corresponding simple chloralides.

Attempts to get crystalline condensation products from mandelic and benzilic acids were fruitless. This is in conformity with the behaviour of aromatic α -hydroxy acids with chloral (Shah and Alimchandani, *loc. cit.*). The reduction of these butylchloralides by zinc dust and acetic acid as in case of simple chloralides (Shah and Alimchandani, *loc. cit.*) has been tried, but no product could be isolated.

EXPERIMENTAL.

Citric Acid Butylchloralide.—Powdered citric acid (25 g.), butylchloral

hydrate (25 g.) were mixed and concentrated sulphuric acid (50 c.c.) slowly added. The substances on shaking dissolved to a brownish solution. The mixture was kept for 24 hours and then poured on to ice; a brownish mass separated which solidified on washing with water. It was collected, dissolved in acetic acid and to the acid solution, concentrated hydrochloric acid added when on keeping a white microcrystalline mass was obtained. Recrystallised from a mixture of ether and petroleum benzine, it separated as rhombic crystals, m. p. $187-88^{\circ}$, yield 8 g. (Found: Cl, 30.20. $C_{10}H_{11}O_7Cl_3$ requires Cl, 30.46 per cent).

The citric acid butylchloralide is soluble in hot water and in common organic solvents except chloroform and petroleum benzine.

Malic Acid Butylchloralide—Malic acid (6 g.), butylchloral hydrate (9 g.) and concentrated sulphuric acid (25 c.c.) were mixed and kept overnight and the reaction mixture worked up as before. The product was crystallised from a mixture of benzene and petroleum benzine, m. p. 139° , yield 2.3 g. (Found: Cl, 36.27. $C_8H_9O_5Cl_3$ requires Cl, 36.52 per cent).

Tartaric Acid Butylchloralide.—Tartaric acid (15 g.), butylchloral hydrate (42 g., 2 mols.) and concentrated sulphuric acid (50 c.c.) were mixed and the mixture was kept for 24 hours, poured into ice-cold water when a pasty mass separated, which on repeated washing with water, hardened. The product, thus obtained, was found to be soluble in all common organic solvents, gelatinous mass separating. The crude product was dissolved in ether and petroleum benzine added, when on keeping a white crystalline mass was obtained, m. p. $82-83^{\circ}$ (mixed m. p. with butylchloral hydrate). The mother-liquor on removal of the solvent gave a substance which was dried on a porous plate and the dry mass crystallised from acetic acid and then from alcohol as small colourless needles, m. p. 156° , yield 2 g. (Found: Cl, 45.55. $C_{12}H_{13}O_8Cl_6$ requires Cl, 45.78 per cent).

The above condensation was repeated with less amount of butylchloral hydrate with no better results. In subsequent work, the excess of the butylchloral hydrate was removed by treating the crude product with hot water.

CHEMISTRY OF SUBSTITUTED RING COMPOUNDS. PART I. SYNTHESIS OF $\alpha\alpha\gamma$ TRIMETHYLCYCLOPENTANONE.

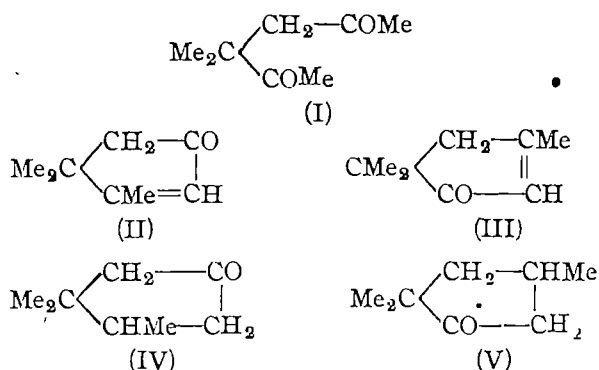
BY MUHAMMAD QUDRAT-I-KHUDA AND SUBASH KUMAR GHOSH.

Synthesis of $\alpha\alpha\gamma$ -trimethylcyclopentanone has been effected from $\alpha\alpha\gamma$ -trimethyladipic acid. The acid has been synthesised by two independent processes. In one of these mesitonic ester has been allowed to react with ethyl bromoacetate in presence of magnesium yielding a lactonic ester. From this the chloro-ester has been obtained which on reduction gives the substituted adipic ester. In the second method mesitonic ester has been condensed with ethyl cyanoacetate and the unsaturated ester, thus prepared, gives the saturated compound which ultimately produces $\alpha\alpha\gamma$ -trimethyladipic ester. The ketone has been found to be identical with that prepared by Wallach by the degradation of dimethyldihydrosophorone.

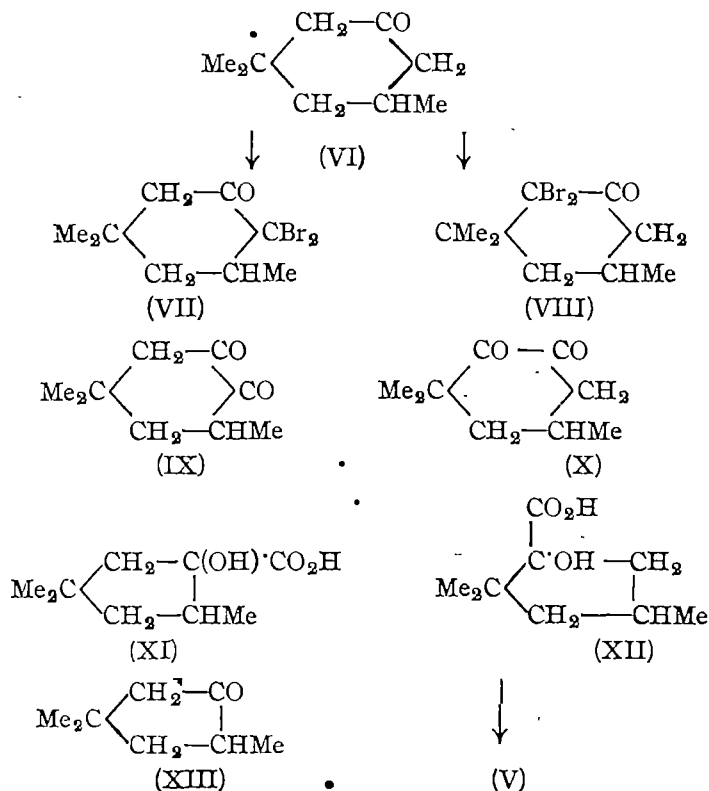
The exact nature of "steric influence" of alkyl substituents on alicyclic rings has not yet been very clearly understood. It is proposed in this series of papers to examine the influence of various alkyl as well as aryl substituents on the formation and stability of some of these ring systems. It is also proposed to examine here incidentally, the influence, if any, that may be brought about in altering the specific character of a definite type of ring compound by different substituents.

It has been observed that by the introduction of methyl groups into a cyclohexane ring, its influence is altered to a marked extent, as has been found in its influence on the tautomeric nature of *p*-methylcyclohexane-1-acetyl-1-malonic acid (Qudrat-i-Khuda and Mukherji, *J. Chem. Soc.*, 1936, 570). It may, therefore, be expected that by an examination of the differently substituted cyclopentanones, as well as cyclohexanones, some valuable informations may be obtained, at least so far as the influence of substituents is concerned. For this purpose several differently substituted cyclopentanones have been synthesised. Of these in the present paper is described the synthesis of $\alpha\alpha\gamma$ -trimethylcyclopentanone (V), some of whose derivatives may give the necessary information.

Wallach prepared two trimethylcyclopentanones; one by the internal condensation of the diketone (I) (*Annalen*, 1916, 408, 204) and subsequent reduction, while the other by the degradation of the dibromide of dihydrosophorone (VI) (*Annalen*, 1918, 414, 328); the former of which he represented by the formula (V) and the latter by the formula (XIII). Both the methods are, however, ambiguous. The first method may yield $\alpha\alpha\gamma$ -trimethylcyclopentanone (V) as well as $\beta\beta\gamma$ -trimethylcyclopentanone (IV) respectively, from the initial condensation products (III) or (II).



The second should also give two ketones, according as the dibromide of dihydro-isophorone is either (VII) or (VIII), which may give the diketone (IX) or (X) and the decomposition of these would lead to the formation of the hydroxy acids (XI) or (XII), which should produce the ketones (XIII) or (V) respectively. The actual experiment, however, gives only one product leaving its identity uncertain.

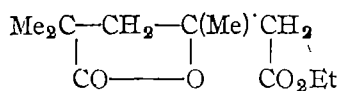


It is not, therefore, surprising that Wallach was led to a wrong conclusion.

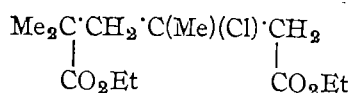
The method employed by us does not leave any room for doubt, regarding the constitution of the ketone (V). From evidences, now accumulated, we are led to suggest that the ketone prepared by Wallach from dihydroisophorone should be represented by the formula (V) and not by (XIII) as suggested by him. By the decomposition of the dibromodihydroisophorone by alkali and subsequent treatment with lead peroxide, Wallach (*loc. cit.*) obtained a ketone that gave a semicarbazone melting at 173°, which is the melting point of the semicarbazone of our ketone (V) (mixed m. p.). Hence, the dibromodihydroisophorone and the diketone should respectively be (VIII) and (X).

The synthesis of $\alpha\gamma$ -trimethyladipic acid has been carried out by two different methods.

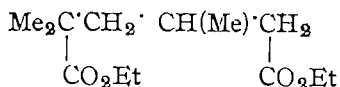
(a) Mesitonic ester prepared by the modification of the method of Lápworth (*J. Chem. Soc.*, 1904, 88, 1219), when acted upon by ethyl bromoacetate in presence of magnesium, gives the lactonic ester (XIV) in good yield. This has been treated with phosphorus pentachloride and the intermediate chloro-acid chloride with absolute alcohol gives ethyl $\alpha\gamma$ -trimethyl- γ -chloroadipate (XV). This on reduction gives the adipic ester (XVI).



(XIV)

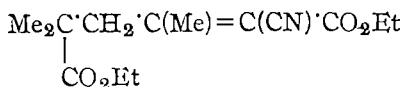


(XV)

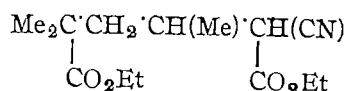


(XVI)

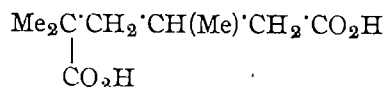
(b) The same ester has also been prepared by condensing mesitonic ester with ethyl cyanoacetate and reducing the unsaturated ester (XVII) with aluminium amalgam when (XVIII) has been obtained.



(XVII)



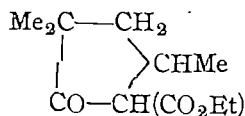
(XVIII)



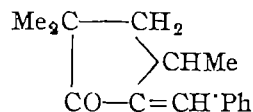
(XIX)

The cyano ester (XVIII) on hydrolysis gives $\alpha\gamma$ -trimethyladipic acid (XIX), and is identical with the acid obtained from the ester (XVI).

The ester (XVI) on being acted upon by an alcoholic solution of sodium ethoxide yields the ketonic ester (XX), which on hydrolysis and decarboxylation gives $\alpha\gamma$ -trimethylcyclopentanone (V). This ketone as is to be expected, yields a characteristic monobenzylidene compound (XXI).



(XX)



(XXI)

EXPERIMENTAL.

Ethyl $\alpha\alpha$ -Dimethylaevulinate (Mesitonic ester).—To a solution of mesityl oxide (98 g.) in boiling alcohol was gradually added a solution of pure potassium cyanide (131 g.) in water (270 c.c.) with frequent shaking. Two distinct layers were at first noticed, but after 7 minutes a very vigorous reaction started, which subsided during 20 minutes, giving a clear solution. It was next cooled in ice and a solution of pure ferrous sulphate (93 g.) in water (180 c.c.) was slowly poured in, with repeated shaking.

The mixture was warmed for another 10 minutes, care being taken that it remained alkaline throughout. The solution was filtered and the residue was thoroughly washed with hot alcohol. The solvent was removed as far as possible, under reduced pressure and the residue was well cooled in ice and acidified with four times its volume of concentrated hydrochloric acid. The reaction was allowed to proceed overnight in the cold. It was then evaporated to dryness on a water-bath and the powdered mass extracted repeatedly with ether. The acid on being dried in a vacuum desiccator gave a pale brown mass consisting of a mixture of mesitonic acid and some mesitylic acid. yield 55 g.

The crude acid was refluxed for 8 hours with absolute alcohol (300 c.c.), $\frac{1}{5}$ of which had been previously saturated with dry hydrogen chloride at 0°. The alcohol was removed under reduced pressure and the ester taken up

in ether, washed with dilute sodium bicarbonate solution, dried and the solvent removed. The remaining oil was fractionated under reduced pressure when a fraction distilled at $115\text{--}16^\circ/30\text{--}32\text{ mm.}$ and weighed 30 g. This on being redistilled boiled at $90^\circ/7\text{ mm.}$ The higher fraction, b.p. $140\text{--}50^\circ/35\text{ mm.}$ solidified in the receiver, yield 18 g.

The first fraction gave a semicarbazone which, when crystallised from methyl alcohol, melted at 165° and was identified to be that of mesitonic ester. (Found : C, 52.2 ; H, 8.3. $\text{C}_{10}\text{H}_{10}\text{O}_3\text{N}$, requires C, 52.4 ; H, 8.3 per cent). The ester when regenerated from the semicarbazone had $d_4^{22.8}$, 0.96095 ; $n_D^{22.8}$, 1.41576, whence $[R_L]_D$, 44.9 (calc. 45.3). This was hydrolysed by aqueous hydrochloric acid when an acid, m.p. 72° , was obtained. It gave a semicarbazone, m.p. 196° . The acid possessed all the properties assigned to it by Pinner (*Ber.*, 1881, 14, 1072 ; 1882, 16, 585) and Lapworth (*J. Chem. Soc.*, 1904, 85, 1219).

The second fraction consisted of the ester of mesitylic acid and gave mesitylic acid on hydrolysis. It was recrystallised from water when it melted at 173° . (Found : C, 56.07 ; H, 7.65. $\text{C}_8\text{H}_8\text{O}_3$ requires C, 56.14 ; H, 7.6 per cent).

Ethyl $\alpha\gamma$ -Trimethylbutyrolactone- γ -acetate (XIV).—Magnesium turnings (4.3 g.) were added to a mixture of mesitonic ester (25.8 g.) and ethyl bromoacetate (25.1 g.) in dry benzene (80 c.c.). A vigorous reaction started after heating the mixture for 5 minutes on a steam-bath. This was allowed to proceed as vigorously as was consistent with safety, and heating on the water-bath was continued for 3 hours when the magnesium dissolved completely. The mass was decomposed with ice and dilute sulphuric acid, and the benzene layer was separated, washed successively with dilute sulphuric acid (10%) sodium hydroxide (10%) and water. It was then dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure (40–45 mm.) on a water-bath. The residual oil on being fractionated gave the lactonic ester, b.p. $148\text{--}50^\circ/7\text{ mm.}$, yield 17 g. The subsequent fraction, b.p. $170\text{--}75^\circ/6\text{ mm.}$ (thick liquid, yield 2 g.), has not yet been investigated.

The lactonic ester on redistillation boiled at $148\text{--}50^\circ/7\text{ mm.}$ It is a colourless mobile liquid, insoluble in water ; $d_4^{31.2}$, 1.0471 ; $n_D^{31.2}$, 1.44355, whence $[R_L]_D$, 54.2 (calc. 54.11). (Found : C, 61.5 ; H, 8.3. $\text{C}_{11}\text{H}_{12}\text{O}_4$ requires C, 61.7 ; H, 8.41 per cent).

Ethyl $\alpha\gamma$ -Trimethyl- γ -chloroadipate (XV).—The lactonic ester (12 g.) was heated with phosphorus pentachloride (26 g.) for 3 hours on a steam-bath when a clear solution was obtained. The whole was then cooled well and poured gradually into well cooled absolute alcohol (75 c.c.) and after

standing overnight, a little water was added to it when an oil separated which was taken up in ether. The ethereal solution was washed successively with dilute sodium carbonate and water, dried over calcium chloride, the solvent removed and the residual oil carefully distilled under reduced pressure from a glycerine bath. The chloro-ester came over at $113-15^{\circ}/5\text{mm.}$, yield 78%. It is a colourless mobile liquid having $d_4^{31.5}$, 1.0381; $n_D^{31.5}$, 1.43913, whence $[R_L]_D$, 70.6 (calc. 70.4). (Found: Cl, 12.45. $\text{C}_{13}\text{H}_{23}\text{O}_4\text{Cl}$ requires Cl, 12.5 per cent).

Ethyl $\alpha\gamma$ -Trimethyladipate (XVI).—Ethyl $\alpha\gamma$ -trimethyl- γ -chloroadipate (20 g.) was dissolved in glacial acetic acid (290 c.c.) and heated on a sand-bath with a reflux condenser. Zinc dust (40 g.) was added to the hot solution in small portions with frequent shaking during 10 hours. Heating was continued for another 10 hours. The solution was decanted off from the lumps of zinc. Any substance adhering to the lumps was washed away with ether. The solution was cooled in ice, just neutralised with caustic soda solution and thoroughly extracted with ether. The ethereal solution was washed with little water, dried and the solvent removed. The ester distilled at $164^{\circ}/44.45\text{ mm.}$, yield 8 g.; when redistilled it came over at $145-146^{\circ}/16\text{ mm.}$ It is a colourless mobile liquid and has d_4^{32} , 0.99383; n_D^{32} , 1.44335, whence $[R_L]_D$, 65.3 (calc. 65.6). (Found: C, 63.6; H, 9.5. $\text{C}_{13}\text{H}_{24}\text{O}_4$ requires C, 63.9; H, 9.8 per cent).

$\alpha\gamma$ -Trimethyladipic Acid (XIX).—Ethyl $\alpha\gamma$ -trimethyladipate (XVI) (8 g.) was hydrolysed with hydrochloric acid (15%, 300 c.c.) by refluxing on a sand-bath for 12 hours. The solution was then evaporated to a small bulk on a water-bath, neutralised with sodium carbonate solution, the unhydrolysed ester removed by ether and the solution was then evaporated almost to dryness, cooled and acidified with concentrated hydrochloric acid. The acid separating was carefully extracted with ether the ethereal extract kept overnight with anhydrous sodium sulphate in contact with animal charcoal. The filtrate on removal of ether, gave a heavy oil which solidified on cooling. It was dried in a desiccator and crystallised from dry ether, m. p. 80° . (Found: C, 57.19; H, 8.2. $\text{C}_9\text{H}_{16}\text{O}_4$ requires C, 57.4; H, 8.5 per cent).

Ethyl α -Cyano $\beta\delta\delta$ -trimethyl- Δ^{α} -butene- $\alpha\delta$ -dicarboxylate (XVII).—This was prepared by some modification of the method of Bardhan (*J. Chem. Soc.*, 1936, 1852). To a mixture of mesitonic ester (114.6 g.) and ethyl cyanoacetate (75.4 g.) were added piperidine (6 c.c.) and anhydrous sodium sulphate (25 g.). After keeping the mixture overnight at the room temperature it was heated on a water-bath for 30 hours, a fresh quantity of sodium sulphate (15 g.) was then added and the heating continued for

another 30 hours. The cold product was poured into water, acidified with dilute hydrochloric acid and the heavy oil taken up in ether. The ethereal solution was washed with aqueous sodium carbonate, dried and fractionated under reduced pressure. At first some unchanged ketonic ester and cyano-ester came over. The unsaturated cyano-ester distilled at $158-64^{\circ}/4$ mm. The mixture of unchanged ketonic ester and cyanoacetic ester was again mixed with 3 c.c. of piperidine and sodium sulphate (10 g.) and treated as before. The process was repeated several times, and the combined high boiling fraction was purified by redistillation under reduced pressure. From the above quantities the total yield was 98 g. When redistilled the unsaturated cyano-ester boiled at $162^{\circ}/4$ mm. It is a colourless heavy oil having d_{4}^{20} , 1.0431; n_D^{20} , 1.45717, whence $[R_L]_D$, -69.68 (calc. 69.4). (Found. C, 62.8; H, 8.05. $C_{14}H_{21}O_4N$ requires C, 62.9; H, 7.86 per cent).

Ethyl α -Cyano- $\beta\delta\delta$ -trimethylbutane- $\alpha\delta$ -dicarboxylate (XVIII).—Aluminium amalgam, prepared from aluminium foil (200 g), was taken in a flask fitted with a tap-funnel and an efficient condenser and covered with moist ether (400 c.c) and the ethereal solution of ethyl α -cyano- $\beta\delta\delta$ -trimethyl- Δ^{α} -butene- $\alpha\delta$ -dicarboxylate (20 g.) was added as quickly as possible; vigorous boiling ensued and the reaction slackened after some time. Water (1 or 2 c.c.) was then added at times to accelerate the reaction. The contents were shaken frequently. The reaction gradually slackened after about 9 hours. The very slow reaction was allowed to proceed overnight. The ethereal solution was decanted and the slimy residue was thoroughly extracted with ether. The ethereal solution was dried over anhydrous magnesium sulphate and on removing the solvent a thick liquid was obtained which was subjected to the same reduction process once again to effect complete reduction. The ester was finally fractionated under reduced pressure when the saturated cyano-ester boiled at $148-52^{\circ}/5$ mm., yield 9 g. On redistillation it was obtained as a thick colourless liquid, b. p. $155-56^{\circ}/8$ mm. It had d_{4}^{22} , 1.0236, n_D^{22} , 1.44033; whence $[R_L]_D$, 69.4 (calc. 69.6). (Found: C, 62.1; H, 8.3. $C_{14}H_{23}O_4N$ requires C, 62.4; H, 8.5 per cent).

$\alpha\gamma$ -Trimethyladipic Acid (XIX).—Ethyl α -cyanotrimethylbutane- $\alpha\delta$ -dicarboxylate (20 g.), mixed with concentrated hydrochloric acid (d 1.21, 400 c.c.) was refluxed for 35 hours. This was then evaporated to a small bulk and neutralised with sodium carbonate solution. The unreacted ester was washed with ether and the aqueous solution was evaporated to dryness. The mass was cooled in ice, acidified with hydro-

chloric acid and extracted five times with ether. The ethereal solution was dried and the solvent removed, when an oily mass was obtained, which solidified on keeping in an evacuated desiccator. On crystallisation from dry ether it melted at 80° and was found to be identical with the acid described before (mixed m. p. 80°).

Ethyl $\alpha\gamma$ -Trimethylcyclopentanone- δ -carboxylate (XX).—Sodium (2.3 g.) was dissolved in absolute alcohol (100 c.c.) and ethyl $\alpha\gamma$ -trimethyladipate (21.5 g.) was added to the well cooled sodium ethoxide solution with constant shaking. The mixture was refluxed for 6 hours on a steam-bath. The product was cooled in ice, decomposed with dilute hydrochloric acid and extracted with ether. After removal of ether, alcohol was removed under reduced pressure. Ethyl $\alpha\gamma$ -trimethylcyclopentanone- δ -carboxylate distilled as a colourless liquid, b. p., $88-90^{\circ}/5$ mm; $d_4^{21.8}$, 0.98559; $n_D^{31.8}$, 1.43882, whence $[R_L]_D$, 52.85 (calc. 52.5), yield 8.5 g. (48.7%). (Found: C, 66.38; H, 8.82. $C_{11}H_{18}O_3$ requires C, 66.7; H, 8.9 per cent). It gives a distinct pink colouration with ferric chloride solution.

$\alpha\gamma$ -Trimethylcyclopentanone (V).—Ethyl $\alpha\gamma$ -trimethylcyclopentanone- δ -carboxylate (8.5 g.) was treated with dilute hydrochloric acid (1:3, 100 c.c.) and refluxed for 2 hours. The acid was neutralised with strong sodium carbonate solution and the oil that separated was taken up in ether and on removing the solvent the residual liquid was fractionated under reduced pressure. The ketone distilled at $65-66^{\circ}/45$ mm.; $d_4^{22.8}$, 0.87660, $n_D^{33.8}$, 1.42426, whence $[R_L]_D$, 36.68 (calc. 36.9); yield 2 g. (45.5%) (Found: C, 75.8; H, 10.9. $C_8H_{14}O$ requires C, 76.19; H, 11.1 per cent). It was found to possess distinct camphoraceous odour. The semicarbazone of the ketone was readily obtained as colourless shining flakes from methyl alcohol. When recrystallised from methyl alcohol it melts at 173° . (Found: C, 58.8; H, 9.2. $C_9H_{17}ON_3$ requires C, 59.0; H, 9.29 per cent). The trimethylcyclopentanone yielded a characteristic monobenzylidene compound (XXI), which when crystallised from petrol melted at $125-26^{\circ}$. (Found: C, 83.78; H, 8.5. $C_{16}H_{18}O$ requires C, 84.1; H, 8.4 per cent).

Wallach's Ketone.—Dibromodihydroisophorone (VII) was prepared by the action of dry bromine (12 c.c.) on a solution of dihydroisophorone (10 g.) in dry carbon disulphide (30 c.c.) The bromination proceeded quite easily with the production of 19 g. of dibromodihydroisophorone. After two crystallisations from methyl alcohol, it melted at 90° (cf. Wallach, *loc. cit.*).

Dibromodihydroisophorone (25 g.) was shaken with a solution of potassium hydroxide (17 g.) in water (667 c.c.) for 6 hours and the diketone was isolated from the solid after acidification with hydrochloric acid by distillation in a current of steam. The diketone (X) separated as a light yellow crystalline solid, m.p. $168-69^{\circ}$ and not at $89-90^{\circ}$ as mentioned by Wallach. (Found: C, 69.7; H, 8.9. $C_9H_{14}O_2$ requires C, 70.08; H, 9.1 per cent). The diketone (10 g.) gave trimethylhydroxycyclopentane-carboxylic acid (XII) on being heated in a sealed tube with 50 c.c. of potassium hydroxide solution (1:2) at about 140° for 4 hours and after usual purification melted at 90° . When treated with lead peroxide as described by Wallach, it gave the ketone (V) which yielded a semicarbazone, m. p. 173° , as in the previous case.

One of us (S.K.G.) begs to thank Sir P.C. Rây, for the award of a fellowship, during the tenure of which this work was completed.

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Received May 15, 1939.

STUDIES IN CHALKONES. PART I. CHALKONES DERIVED FROM RESACETOPHENONE AND ITS DIMETHYL ETHER.

BY JAGRAJ BEHARI LAL.

Resacetophenone dimethyl ether has been condensed with *o*-vanillin, isovanillin, 5- and 6-methoxysalicylaldehyde, and resacetophenone with *o*-vanillin to yield corresponding hydroxymethoxychalkones. Attempt has been made to determine the optimum condition for these condensations.

On account of the fact that the resorcinol nucleus occurs in the vast group of natural flavone, isoflavone, flavonol and chalcone colouring matters, it would appear to be of interest to synthesise compounds of this type. The most easily synthesised are the chalkones, a few of which have been prepared by previous workers by condensation of resacetophenone and its mono- and dimethyl ethers with aldehydes (Perkin and Hummel, *J. Chem. Soc.*, 1904, **86**, 1459; Perkin, Robinson and Turner, *J. Chem. Soc.*, 1908, **93**, 1109; Göschke and Tambor, *Ber.*, 1911, **44**, 3502, 1912, **45**, 186; Bargellini and Lydia Monti, *Gazzetta*, 1914, *ii*, **44**, 25; Tambor, *Ber.*, 1916, **49**, 1704; Shinoda, *J. Pharm. Soc. Japan*, 1928, **48**, 214; Shinoda, Sato and Kawangoe, *J. Pharm. Soc. Japan*, 1929, **49**, 123; Hattori, *Acta Phytochim.*, 1932, **6**, 131; Russell, *J. Chem. Soc.*, 1934, 218; Mahal, Rai and Venkataraman, *J. Chem. Soc.*, 1935, 866; Saiyad, Nadkarni and Wheeler, *J. Chem. Soc.*, 1937, 1737; Nadkarni and Wheeler, *ibid.*, 1938, 1320).

In the present communication resacetophenone dimethyl ether has been condensed with *ortho*-vanillin, 5- and 6-methoxysalicylaldehyde, and with isovanillin and resacetophenone with *ortho*-vanillin, giving the corresponding hydroxymethoxychalkones. An attempt has also been made to determine the optimum conditions for condensing resacetophenone and its dimethyl ether with some aldehydes in order to obtain the maximum possible yields of the chalkones, specially in view of the fact that the reaction is reversible (*cf.* Schriner and Kurosawa, *J. Amer. Chem. Soc.*, 1930, **52**, 2538), the equilibrium being dependent on the concentration of alkali and on the temperature, these two conditions varying within wide limits from one chalcone to another.

EXPERIMENTAL.

Pure resacetophenone was prepared by the following modification of

the original method due to Nencki and Sieder (*J. pr. Chem.*, 1881, **23**, 147). Fused zinc chloride (55 g.) was dissolved in hot glacial acetic acid (60 g.), freshly distilled over fused sodium acetate, and dry resorcinol (40 g.) added and the mixture heated on the sand-bath until the temperature of the mixture was 144° when the heating was discontinued. Robinson and Shah (*J. Chem. Soc.*, 1934, 1494) by treating the cold reaction mixture with a mixture of equal volumes of concentrated hydrochloric acid and water obtained moderately pure resacetophenone, m. p. 133-140° (yield 91%), while the present author obtained 72% of resacetophenone, m. p. 144-45°, by crystallising the product from hot water.

Methyl alcohol used in the followig condensations was freed from traces of formaldehyde by distillation over silver nitrate.

2:4-Dimethoxyphenyl-3'-hydroxy-4'-methoxystyryl ketone.—(a) A mixture of resacetophenone dimethyl ether (3.2 g.), isovanillin (2.5 g.), methyl alcohol (10 c. c.) and 30% methyl alcoholic caustic potash (10 c. c.) was heated to boiling on the water-bath, when the mixture quickly became deep red. After keeping at 50-60° for 24 hours, the mixture was diluted with water, boiled to remove methyl alcohol, and extracted with ether to remove any unchanged resacetophenone dimethyl ether. The viscous precipitate obtained on acidification with dilute acetic acid was washed with water and thrice crystallised from small quantities of hot alcohol in lemon-yellow stout prismatic needles, m. p. 115°, yield 3.9 g. (73.6%). (Found : C, 68.8 ; H, 5.7. $C_{18}H_{18}O_3$ requires C, 68.8 ; H, 5.7 per cent).

It is readily soluble in cold methyl and ethyl alcohol, acetone, ethyl acetate, benzene, chloroform, carbon disulphide and carbon tetrachloride, and less so in ether and petroleum ether. It dissolves in alkalis to a deep yellow or orange-red solution, and with concentrated sulphuric acid and hydrochloric acid gives a deep orange red colouration; but there is no colouration with alcoholic ferric chloride.

(b) A mixture of resacetophenone dimethyl ether (3.6 g.), isovanillin (3 g.) methyl alcohol (20 c.c.) and 25% methyl alcoholic caustic potash (50 c.c.) was heated under reflux on the water-bath for 1 hour when it quickly became bright red, but no precipitate separated. After keeping for 12 hours at 40-45°, the red solution was diluted with water and acidified with dilute sulphuric acid giving a yellow crystalline precipitate of the chalkone (5.5 g; 88.7%).

2 : 4-Dimethoxyphenyl- 2'- hydroxy- 6'- methoxystyrylketone.—(a) A mixture of resacetophenone dimethyl ether (3.6 g.), 6'-methoxysalicyl aldehyde (3 g.), methyl alcohol (30 c. c.) and 50% aqueous caustic potash (20 c. c.) was heated to boiling on the water-bath for 10 minutes

and then kept at 50-55° for 24 hours, when a bulky red precipitate of the potassium derivative of the chalkone separated. It was filtered, washed with a little methyl alcohol and its aqueous solution acidified with dilute sulphuric acid, when a lemon-yellow precipitate (5.1 g. and 0.2 g.) on acidification (after dilution) of the filtrate from the potassium salt was obtained. It was crystallised once from dilute ethyl alcohol (greenish yellow prisms), twice from hot benzene and finally from ethyl acetate as yellow leaflets or plates, m. p. 116.5°. (Found in sample dried at 80° under high vacuum: C, 68.7; H, 5.8. $C_{18}H_{18}O_3$ requires C, 68.8; H, 5.7 per cent). It is fairly soluble in all organic solvents in the cold. It gives a deep red colouration with concentrated sulphuric and hydrochloric acid and an intense green colouration with alcoholic ferric chloride.

The results of other experiments are given in Table I.

TABLE I.

Caustic potash.	Duration of heating on the water-bath.	Kept at	Yield
20 C. c. of 50% aq	10 min.	50-55° for 24 hr	5.3 g. (82.3%)
40 C. c. of 40% methyl alcoholic	20	50° „ 12	3.4 g. (55.8%)
40 C. c. of 25% „ „	30	50° „ 12	2.6 g. (41.9%)
30 C. c. of 25% „ „	30	20-22° „ 24	2.3 g. (37.0%)

2:4-Dimethoxyphenyl-2'-hydroxy-3'-methoxystyrylketone.—A mixture of resacetophenone dimethyl ether (3.6 g.), *o*-vanillin (3 g.), methyl alcohol (20 c.c.) and 50% aqueous caustic potash (20 c.c.) was heated for 10 minutes on the water-bath, when the potassium derivative of the chalkone separated as bulky glistening red needles. The mixture was then kept at 50-55° for 24 hours. The potassium salt was filtered and decomposed in aqueous suspension with dilute hydrochloric acid. The dried lemon-yellow crystalline product (4.9 g., 79.6%) was recrystallised from boiling alcohol as bright yellow prisms, m.p. 117°. (Found in sample dried at 80° in high vacuum: C, 68.6; H, 5.8. $C_{18}H_{18}O_3$ requires C, 68.8; H, 5.7 per cent). It is moderately soluble in cold ethyl and methyl alcohol and ethyl acetate, and sparingly in cold benzene, chloroform, carbon tetrachloride, ether and petroleum ether. It gives a deep red colouration with sulphuric and hydrochloric acid, and with alcoholic ferric chloride, a green colouration.

The results of other experiments are given in Table II.

TABLE II.

Caustic potash	Duration of heating on the water-bath	Kept at	Yield
20 C.c. of 50% aq.	10 min.	50-55° for 24 hr.	4.9 g. (79.6%)
40 C.c. of 25% methyl alcoholic	20	50-55° for 12	5.4 g. (87.0%)
50 C.c. of 40% methyl alcoholic	20	50° for 12	4.8 g. (75.13%)

2 : 4-Dimethoxyphenyl-2'-hydroxy-5'-methoxystyrylketone.—A mixture of resacetophenone dimethyl ether (3.6 g.), 5-methoxysalicylaldehyde (3 g.), methyl alcohol (30 c.c.) and 50% aqueous caustic potash (20 c.c.) was heated for 10 minutes on the water-bath and then kept at 50–60° for 12 hours. The bulky red crystalline precipitate of the potassium salt was filtered; washed with a little alcohol, dissolved in water and acidified; the orange-yellow precipitate was washed and dried (5.1 g., 82.2%). It crystallised from aqueous alcohol and also from benzene as brownish yellow needles, m.p. 129°. (Found: C, 68.7; H, 5.8. $C_{18}H_{18}O_5$ requires C, 68.8; H, 5.7 per cent). It is readily soluble in cold methyl and ethyl alcohol and ethyl acetate and slightly in benzene, carbon tetrachloride, chloroform, petroleum ether and ether. It gives with concentrated sulphuric and hydrochloric acid a cherry-red colouration and none with alcoholic ferric chloride.

2 : 4-Dihydroxyphenyl-2'-hydroxy-3'-methoxystyrylketone.—A mixture of resacetophenone (3 g.), *ortho*-vanillin (3 g.), methyl alcohol (40 c.c.) and 50% aqueous caustic potash (40 c.c.) was heated on the water-bath at 60–65° for 24 hours, when it became deep red in colour and the red potassium derivative of the condensation product crystallised out. The reaction mixture was cooled, filtered and treated with dilute acetic acid giving a gummy yellow mass, which solidified and crumbled to a fine yellow powder on standing. It was filtered, well washed and dried. The filtrate from the potassium salt similarly gave the chalkone (yield 3.6 g., 63.8%). The acid filtrate on concentration gave a dirty white crystalline substance (1 g.) which is under examination.

The chalkone was dissolved in the minimum amount of hot methyl alcohol and water added to slight turbidity, when it separated as tiny bright yellow needles, the mother-liquor on concentration yielding more of the substance. It was recrystallised from alcohol as bright yellow needles, m.p. 211°. (Found in sample dried at 100°: C, 67.0; H, 5.1. $C_{18}H_{14}O_5$ requires C, 67.1; H, 4.49 per cent).

It is readily soluble in cold ethyl and methyl alcohol, moderately so in ethyl acetate, and almost insoluble in benzene, chloroform, carbon tetrachloride, petroleum ether and ether. Concentrated sulphuric acid gives a cherry-red colouration, and hydrochloric acid none in the cold, but a red colouration on warming, and alcoholic ferric chloride, a dark greenish brown colouration.

The results of other experiments are recorded in Table III.

TABLE III.

Caustic potash.	Duration of heating on the water-bath	Kept at		Yield.
40 C.c. of 50% aq.	0 hr.	60-65°	for 24 hr.	3.6 g. (63.8%)
60 C.c. of 25% methyl alcoholic	0	60°	24	4.5 g. (83.3%)
40 C.c. of 25% methyl alcoholic	4	20-22°	24	1.1 g. (19.5%)
60 C.c. of 25% methyl alcoholic	2	20-22°	24	2.15 g. (35.2%)

The author wishes to convey his heartiest thanks to Dr. S. Dutt for his kind interest in the investigation.

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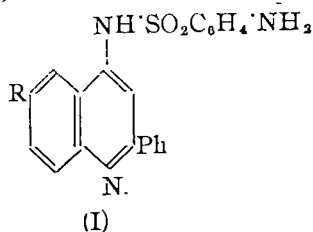
Received January 19, 1939.

ON 2-PHENYL-4-AMINOQUINOLINE DERIVATIVES.

BY U. P. BASU AND P. K. DAS-GUPTA

Certain 2-phenyl-4-(dialkylamino-4'-sulphonamidophenyl-, and 4'-amidobenzene-sulphonyl-) aminoquinolines have been described. The yield of the condensation product is invariably poor.

The recent researches in chemotherapy have revealed that the dialkyl-aminoalkylamine chains play a considerable part in the development of the therapeutic activity in quinoline and acridine derivatives. Similarly sulphanilamide and its derivatives have been found to possess definite bacteriostatic action against various coccal infections. It was, accordingly, considered to be of interest to study the class of compounds formed by the fusion of the above groupings to the γ -position of 2-phenylquinoline, the 4-carboxyl derivative of which is also largely used in medicine. It may be mentioned that 2-phenyl-, and 2-phenyl-6-methoxy-4-(4'-aminobenzenesulphonyl)-aminoquinolines (I, R = H or MeO) are expected to have some therapeutic importance since the replacement of one hydrogen atom of sulphonamido group of *p*-aminobenzenesulphonamide often widens the range of activity of the drug (*cf.* Whitby, *Lancet*, 1937, *i*, 1517; 1938, *i*, 1210; Grütz, *Deut. Med. Woch*, 1937 **33**, 1932; Domagk, *Klin. Woch.*, 1937, **16**, 1412).



EXPERIMENTAL.

2-Phenyl-4-(β -diethylaminoethyl)-aminoquinoline.—A mixture of 2-phenyl-4-aminoquinoline (4.4 g.), prepared according to John (*Ber.*, 1926, **59**, 1448), diethylaminoethyl bromide (4 g.), anhydrous potassium carbonate (2 g.) and a trace of copper powder in dry xylene was heated under reflux for 12 hours. The mixture was then extracted with dilute hydrochloric acid and the extract was rendered neutral to congo-red with potassium carbonate. It was then extracted with ether to remove any unchanged 2-phenyl-4-aminoquinoline and the aqueous portion was treated with excess of potassium carbonate when the product separated out as an oily mass. This was then dissolved

in ether, the ethereal solution dried over anhydrous potassium carbonate and ether removed and the residue distilled under reduced pressure. The portion boiling between $272-76^{\circ}/6$ mm., was collected. (Found: N, 13.2. $C_{21}H_{25}N_3$ requires N, 13.16 per cent). It is a viscous yellow liquid, n_D^{25} , 1.6510. The base readily forms an extremely hygroscopic hydrochloride and a picrate. The latter was crystallised from a large volume of alcohol in clusters of yellow needles, m.p. $238-39^{\circ}$. (Found: N, 16.08. $C_{21}H_{25}N_3$, $2C_6H_3O_7N_3$ requires N, 16.21 per cent).

2-Phenyl-4-(γ -diethylaminopropyl)-aminoquinoline.— 2-Phenyl-4-aminoquinoline and diethylaminopropyl chloride in molecular proportions were heated under reflux with potassium carbonate and copper in xylene for 24 hours. The product was isolated as described previously, b.p. $265-70^{\circ}/6$ mm. (Found: N, 12.7. $C_{22}H_{27}N_3$ requires N, 12.6 per cent). It is a yellow viscous liquid and forms a hygroscopic hydrochloride. The picrate separated from alcohol in fine yellow needles, m.p. 234° . (Found: N, 16.05. $C_{22}H_{27}N_3$, $2C_6H_3O_7N_3$ requires N, 15.93 per cent).

2-Phenyl-4-(δ -diethylaminobutyl)-aminoquinoline.— A mixture of 2-phenyl-4-chloroquinoline (1 part, Knorr and Fertig, *Ber.*, 1897, 30, 938), diethylamino-*n*-butylamine (1.1 part) in dry amyl alcohol was heated at $110-20^{\circ}$ for 12 hours after the addition of potassium carbonate and copper powder. Amyl alcohol and the unreacted butylamine derivative were removed by steam, and the residual mass was treated with dilute acetic acid in which the chloroquinoline was insoluble. The acetic acid solution was then filtered and the filtrate was neutralised with potassium carbonate, and the whole was then extracted with ether. The ethereal solution on drying over anhydrous potassium carbonate was concentrated to a viscous mass. This was redissolved in dilute acetic acid and the process was repeated. The yield of the crude condensation product was poor and the substance was isolated as picrate which was recrystallised from alcohol in fine yellow microscopic needles, m.p. 203° . (Found: N, 15.74. $C_{23}H_{29}N_3$, $2C_6H_3O_7N_3$ requires N, 15.65 per cent). The base liberated from the picrate with alkali was extracted with ether. The residue from ether, dried over liquid paraffin and phosphorus pentoxide *in vacuo*, was a deep yellow thick liquid. (Found: N, 12.02. $C_{23}H_{29}N_3$ requires N, 12.1 per cent).

2-Phenyl-4-(δ -diethylamino- α -methylbutyl)-aminoquinoline.— δ -Diethylamino- α -methylbutylamine on being heated with 2-phenyl-4-chloroquinoline at $150-60^{\circ}$ for 20 hours gave the phenylaminoquinoline derivative as a viscous mass. This was purified by dissolving in acetic acid and precipitating with potassium carbonate. This viscous mass gave a picrate, m.p. 162° , after crystallisation from alcohol. (Found: N, 15.75. $C_{24}H_{31}N_3$, $2C_6H_3O_7N_3$ requires N, 15.4 per cent).

2-Phenyl-6-methoxy-4-(γ -diethylaminopropyl)-aminoquinoline. — 2-Phenyl-6-methoxy-4-aminoquinoline, prepared from 6-methoxy-atophan as prescribed by John (*loc. cit.*) melted at 159° . (Found: N, 11.35. $C_{16}H_{14}ON_2$ requires N, 11.2 per cent). John and Lukas (*J. pr. Chem.*, 1931, ii, 130, 314) gave m.p. 143° .

The above quinoline (5 g.) and diethylaminopropyl chloride (3 g.) were heated together at $160-70^{\circ}$ for 12 hours. It was then mixed with an aqueous solution of potassium carbonate (1.5 g.) and the whole was subjected to steam distillation for about 1 hour. The mixture was then cooled and extracted with ether. The ethereal solution was shaken with dilute hydrochloric acid and the acid extract was subsequently treated with excess of potassium carbonate. The heavy oil that separated out, was collected and purified in the usual way. It is a very high boiling thick liquid difficult to distil. The picrate of the compound crystallised from alcohol in fine yellow needles with greenish tinge, m.p. 185° . (Found: N, 15.46. $C_{23}H_{20}ON_3$, $2C_6H_3O_7N_3$ requires N, 15.35 per cent).

2-Phenyl-6-methoxy-4-(δ -diethylamino-n-butyl)-aminoquinoline. — 2-Phenyl-6-methoxy-4-chloroquinoline, m. p. 109° (*cf.* John and Lukas, *loc. cit.*), and diethylaminobutylamine in molecular proportions gave the condensation product as a deep yellow viscous mass. After purification by the usual method, the picrate was prepared as yellow needles, m.p. 192° , after crystallisation from alcohol. (Found: N, 15.3. $C_{24}H_{21}ON_3$, $2C_6H_3O_7N_3$ requires N, 15.1 per cent).

2-Phenyl-4-(4'-sulphonamidophenyl)-aminoquinoline. — 2-Phenyl-4-chloroquinoline (2.4 g.) and *p*-aminobenzenesulphonamide (1.6 g.) were heated together with a little copper powder at $160-170^{\circ}$ for 16 hours. The reaction mixture was dissolved in boiling alcohol, filtered, and cautiously basified with dilute ammonia. The solid was collected, washed with hot water and finally crystallised from alcohol (charcoal) in fine colourless needles, m. p. 250° . (Found N, 11.29; S, 8.0. $C_{21}H_{18}O_2N_3S$ requires N, 11.17; S, 8.5 per cent).

2-Phenyl-4-(4'-sulphondiethylamidophenyl)-aminoquinoline. — 2-Phenyl-4-chloroquinoline (2 g.) and *p*-aminobenzenesulphondiethylamide (3 g.) under the above conditions (period of heating 10 hours) afforded the condensation product which crystallised from alcohol in colourless needles, m. p. 144° . (Found: N, 10.0. $C_{25}H_{25}O_2N_3S$ requires N, 9.8 per cent). It is more soluble in alcohol and is also moderately soluble in ether and benzene.

2-Phenyl-4-(4'-amidobenzenesulphonyl)-aminoquinoline. — 2-Phenyl-4-aminoquinoline dissolved in benzene was mixed with a solution of

4-acetaminobenzenesulphonyl chloride in benzene, and the mixture was subsequently heated under reflux for 4 hours. After standing overnight, the benzene solution was decanted off and the solid mass formed was dissolved in alcohol and the solution was then neutralised with dilute ammonia avoiding rise of temperature. The substance crystallised on scratching and was recrystallised from dilute alcohol in colourless needles, m. p. 297° . (Found : N, 10.34; S, 7.55. $C_{23}H_{19}O_3N_3S$ requires N, 10.07; S, 7.67 per cent). The substance is soluble in excess of ammonia and does not give a diazo reaction in the cold.

The above acetyl derivative (2 g.), dissolved in rectified spirit (20 c.c.) and hydrochloric acid (d 1.16, 1.5 c. c.) was heated for 2 hours on the steam-bath. The solution was concentrated, cooled and just neutralised with dilute ammonia. 2-Phenyl-4-(4'-amidobenzenesulphonyl)-aminoquinoline slowly separated out and was crystallised from dilute alcohol in microscopic needles, m. p. 293° . (Found : N, 11.5. $C_{21}H_{17}O_4N_3S$ requires N, 11.2 per cent). The substance is soluble both in acids and alkalis and gives a diazo reaction.

2-Phenyl-6-methoxy-4-(4'-amidobenzenesulphonyl)-aminoquinoline.—2-Phenyl-6-methoxy-5-aminoquinoline and 4-acetaminobenzenesulphonyl chloride were separately dissolved in hot dimethylaniline, mixed and then heated in an oil-bath at 150° for 1 hour. The dimethylaniline was decanted off. The separated solid was dissolved in alcohol, treated with charcoal, filtered and basified with ammonia. On concentration, the solution afforded 2-phenyl-6-methoxy-4-(4'-acetaminobenzenesulphonyl)-aminoquinoline which was finally crystallised from alcohol in colourless needles, m. p. 268° . (Found : N, 9.27; S, 6.95. $C_{24}H_{21}O_4N_3S$ requires N, 9.4; S, 7.18 per cent). It is soluble in excess of ammonia and this ammoniacal solution on heating hydrolyses to 2-phenyl-6-methoxy-4-aminoquinoline, identified by mixed m. p.

The above acetaminobenzenesulphonyl derivative was dissolved in absolute alcohol (25 c. c.) and refluxed on a water-bath for 1 hour after adding 4 c. c. of 5N-hydrochloric acid. The solution was concentrated, cooled and just neutralised with ammonia. The crystals of 2-phenyl-6-methoxy-4-(4'-aminobenzenesulphonyl)-aminoquinoline were collected, washed with water and recrystallised from alcohol in colourless needles, m. p. 268° . (Found : N, 10.48. $C_{22}H_{19}O_3N_3S$ requires N, 10.37 per cent).

VISCOSITY OF NON-IDEAL BINARY LIQUID SYSTEMS.

By M. K. SRINIVASAN.

An equation on the lines of Kendall and Munroe's cube root equation has been proposed and shown to work as satisfactorily as other equations on eleven binary liquid systems whose η - c curves either sag or show maxima or minima.

Various equations have been proposed from time to time expressing the viscosity of binary liquid mixtures as a function of composition, *e.g.*, the cube root equation; $\eta^{\frac{1}{3}} = \eta_1^{\frac{1}{3}}x_1 + \eta_2^{\frac{1}{3}}x_2$, (Kendall and Munroe, *J. Amer. Chem. Soc.*, 1917, **39**, 1787) and the equations of Macleod (*Trans. Faraday Soc.*, 1923, **19**, 17) and Spells (*ibid.*, 1936, **32**, 530). The cube root equation represents some ideal systems quite satisfactorily but fails in the case of some others. It was thought desirable to see, if by taking into account the variations of density that occur on mixing two liquids, the cube root equation may not be made to represent the viscosity of non-ideal liquid mixtures such as those whose η - c curves show maxima, minima, etc. Of the various modifications tried, the following form was found to meet with some success:

$$\eta^{\frac{1}{3}} = \frac{\rho_0}{100} \left(\eta_1^{\frac{1}{3}} v_1 + \eta_2^{\frac{1}{3}} v_2 \right) \left(\frac{\rho_0}{\rho_c} \right)^m$$

where η , η_1 and η_2 are the viscosities of the mixture and pure components, v_1 and v_2 , volumes of the pure components in 100 grams of the mixture, ρ_0 , the observed density of the mixture, ρ_c , the density calculated from the formula $\frac{100}{v_1 + v_2}$, and m , a constant. The results are summarised in the

following table for eleven mixtures. The data for the first five mixtures were obtained from the work of Dunstan and his co-workers (*J. Chem. Soc.*, 1907, **91**, 1728), for 6 and 7 from Baker's paper (*ibid.*, 1912, **101**, 1409, for 8 and 9 from Bramley's (*ibid.*, 1916, **109**, 10) for 10 from Macleod's (*loc. cit.*) and for the last from Srinivasan and Prasads' paper (*Trans. Faraday Soc.*, 1938, **34**, 1139).

TABLE I.

Mixture.	Nature of curve.	Value of m .	Percentage deviation. Present equation.	Macleod's equations (where known).
Picoline-water	Maximum	13.5	14.8	9.1
Pyridine-water	"	16	9.9	5.4
Lutidine-ethyl alcohol	Maximum and minimum	2.5	1.7	2.06
Pyridine-benzene	Almost linear	4.5	2.8	
Pyridine-ethyl alcohol	Minimum	-3.4	3.9	
Methyl alcohol-ethyl ether	Very slight sag	1	4.8	
Ethyl alcohol-phenetole	Minimum	-18	4.2	
Phenol-aniline	Maximum	24	10	
Phenol-acetone	Indefinite	-18.5	5.3	
Ethyl alcohol-water	Maximum	9	18.0	18.8
Acetic acid-water	"	5	21.4	16*

It may be seen from the last two columns that the deviations in the present case are generally of the same order as in Macleod's two constant equation. It would appear thus that the factor $\left(\frac{\rho_0}{\rho_c}\right)^m$ accounts at least in some measure, for the changes in volume or field of force surrounding the molecules, which are the causes for effecting non-ideal conditions. The rather high values for ' m ' in some cases may not be surprising when one considers that even for pure liquids, the forces of attraction between the centres of molecules vary inversely as the eighth power (at least) of their distance (Edser, *Fourth Report of Colloid Chemistry*, D.S.I.R., 1922; Newton Friend, *Trans. Faraday Soc.*, 1938, **34**, 813). However, it is not possible to attach any theoretical significance to the constant.

In the following tables detailed results are given for two of the above mentioned mixtures. They are chosen as being typical of the best (Table III) and the worst (Table II) fit obtained with the equation.

* For different temperatures.

TABLE II.

Ethyl alcohol—water (10°).

Percentage by wt. of alcohol.	Density.	η (obs.).	η (calc.).	Percentage Recent equation.	Percentage Spell's equation.	error Macleod's equation.
0	0.99973	0.01308	0.01308	0	0	0
10	0.98393	0.02179	0.01786	18.0	18.1	18.8
20	0.97252	0.03165	0.02648	16.3	14.2	17.8
30	0.95977	0.04050	0.03594	11.2	6.1	8.1
40	0.94238	0.04390	0.04215	4.0	0.2	0
50	0.92162	0.04180	0.04328	3.5	5.2	3.3
60	0.89927	0.03770	0.04087	8.4	7.1	2.6
70	0.87602	0.03268	0.03605	10.3	7.1	0
80	0.85197	0.02710	0.02987	10.2	0.2	3.3
90	0.82654	0.02101	0.02277	8.4	4.4	2.9
100	0.79784	0.01466	0.01466	0	0	0

TABLE III.

Lutidine—ethyl alcohol (25°).

Percentage by wt. of lutidine.	Density.	η (obs.)	η (calc.).	Percentage Present equation.	Percentage Spell's equation.	error Macleod's equation.
0	0.79043	0.011536	0.011535	0	0	0
9.97	0.80382	0.011499	0.011459	0.35	0.77	0.34
19.88	0.81972	0.011643	0.011702	0.51	0.20	0
39.77	0.85142	0.012023	0.011890	1.1	2.02	1.19
59.70	0.88029	0.011328	0.011335	0.06	0.6	0
79.43	0.90743	0.010204	0.010336	1.3	1.23	2.06
90.55	0.92101	0.009686	0.009518	1.74	1.75	1.92
100	0.93218	0.0087766	0.0087766	0	0	0

I am grateful to Professor B. Prasad for suggesting this piece of work and helping me throughout. My thanks are also due to the Government of Orissa for the grant of a scholarship.

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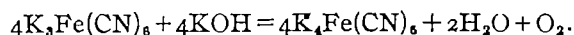
Received May 18 1939.

A NOTE ON THE ACTION OF STRONG SOLUTIONS OF POTASSIUM FERRICYANIDE.

By EDWARD BARNES.

An alkaline solution of $\text{K}_3\text{Fe}(\text{CN})_6$ is a well known oxidising agent, but it does not appear to have been recorded that if the alkali is sufficiently strong, free oxygen is obtained.

On adding 50 c.c. of aqueous KOH solution (1:1) to 5 g. of $\text{K}_3\text{Fe}(\text{CN})_6$ in 10 c.c. of water, the $\text{K}_3\text{Fe}(\text{CN})_6$ is mostly thrown out of solution as an orange-red precipitate, which floats owing to the gas disengaged on its surface. At room temperature (30°), the reaction is slow but is almost complete after being left overnight. The gas evolved contains about 93.5% of oxygen, the rest being nitrogen. On heating to 100° the effervescence becomes fairly brisk, the reaction being complete in about an hour, the gas contains approximately 95% of oxygen. The maximum volume of gas obtained from 5 g. of $\text{K}_3\text{Fe}(\text{CN})_6$ was 79.5 c.c. at N.T.P. There remains after the reaction a pale yellow liquid with a cream-coloured crystalline precipitate. The precipitate consists of slender prisms which do not lose weight appreciably on heating to 100° . It dissolves in water giving a clear solution showing the reactions of a ferrocyanide, and which, on concentration, deposits the characteristic tablet-shaped crystals of $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$. The precipitate appears, therefore, to be anhydrous $\text{K}_4\text{Fe}(\text{CN})_6$. The alkaline solution from the reaction contains a little $\text{K}_3\text{Fe}(\text{CN})_6$ but no $\text{K}_4\text{Fe}(\text{CN})_6$. These results suggest that the main reaction may be represented by the following equation,



With saturated KOH solution the reaction is slower, probably owing to the greater insolubility of $\text{K}_3\text{Fe}(\text{CN})_6$ in this solution. With equal volumes of the $\text{K}_3\text{Fe}(\text{CN})_6$ and KOH solutions, oxygen is evolved, but after long heating the solution remains brown. When the volume of KOH is reduced to one-fifth the volume of the $\text{K}_3\text{Fe}(\text{CN})_6$, there is no noticeable evolution of gas in the cold, and only slight effervescence on heating. With 50 c.c. of NaOH solution (1 NaOH : 1.5 water) and 5 g. of $\text{K}_3\text{Fe}(\text{CN})_6$ in 10 c.c. of water, the $\text{K}_3\text{Fe}(\text{CN})_6$ is precipitated but very slight

effervescence takes place in the cold; at 100° a slow evolution of gas containing 97-98% oxygen occurs. The resulting cream-coloured precipitate consists of hexagonal plates.

That the volume of oxygen obtained is appreciably less than the theoretical amount, 85 c.c., and the presence of some nitrogen in the gas, suggest that a secondary reaction occurs in which some (CN) groups are oxidised. But as no $\text{Fe}(\text{OH})_3$ is formed when the alkali is strong, it appears that no $[\text{Fe}(\text{CN})_6]'''$ radicals are completely broken up.

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Received May 15, 1939.

REVIEW

An Introduction to Crystal Chemistry.—By R. C. EVANS, M.A., PH.D., D.Sc. DEMONSTRATOR IN THE DEPARTMENT OF MINEROLOGY AND PETROLOGY, UNIVERSITY OF CAMBRIDGE. PUBLISHED BY THE UNIVERSITY PRESS, CAMBRIDGE, 1939. PRICE 18 SH. MEDIUM 8vo. PP. 388.

Crystal Chemistry may be defined as the study of relationship between crystal structure and the chemical composition and properties of substances. Though many excellent reviews and articles on the subject by many eminent workers have appeared from time to time within the last decade, the present volume appears to be the first attempt in English to present the subject in the form of a text-book for students of chemistry.

Since the development of the technique of the analysis of crystal structure by X-rays, a study of the internal structure of a wide range of crystalline materials, both inorganic and organic, has been made with the result that many general principles of great significance to chemistry and other allied branches of science have emerged. In the present volume a critical survey of the application of these general principles has been attempted and the author deserves congratulation for the success he has achieved.

The book has been written in an attractive and simple style, and will not fail to create an active interest of the reader in the subject. Both the beginners and advanced students of chemistry in the University are sure to derive much benefit from its study. Many of our conceptions regarding chemical compounds and their constitution in the solid state, based on purely chemical evidences, will need drastic revision in the light of newer knowledge derived from structure analysis.

The book is divided into two parts.. In Part I—the Crystal Lattice, an account of the nature of interatomic binding force dealing with ionic, homopolar, metallic and van der Waal's bond and a classification of crystal structure have been given. This is followed by a chapter on quantitative lattice theory in which quantum theory of the van der Waal's bond, lattice theory of ionic crystals, quantum theory of the homopolar bond, theory of the metallic state and a general theory of the solid state have been briefly discussed. Part II deals with the systematic crystal chemistry involving the structures of the metallic elements, alloy system, homopolar compounds,

ionic compounds of various types and those of molecular compounds. No attempt at an exhaustive treatment has, however, been made but only typical cases of each class suited to illustrate the general principles of crystal chemistry have been described. A generous provision of reference materials together with the bibliography added at the end of the book, will enable the reader to make a further and detailed study of any part of the subjects.

There are, however, certain chapters in the book, *e g.*, the theory of the metallic state and a general theory of the solid state, which it will be difficult for the beginners to follow without previous preparation. Here they will, however, find a ready guide in the complete list of references supplied.

There are very few mistakes, mostly of the typographical nature, in the book and it is expected that they will be corrected in the next edition. The reviewer may make here a mention of the following —

On p. 254, l. 11 from the top, the word "pyrosulphate" is evidently a misprint for "pyrosulphite."

On p. 258, l. 13 from the top, the word "chlorates" should be replaced by "perchlorates."

On p. 342, l. 4 from the bottom, the word "molecular" is obviously a misprint for "molecule."

The print and get-up of the book are excellent and attractive.

The reviewer cannot too strongly recommend the present volume to the post-graduate students of Indian Universities.

P. R.

COLOUR AND MAGNETIC PROPERTIES OF MANGANOUS SULPHIDE.

By S. S. BHATNAGAR, BRAHM PRAKASH AND JARNAIL SINGH.

In this work the magnetic susceptibilities of the green and the pink varieties of manganous sulphide have been measured on a Gouy's balance. It is shown that the cubic B₃ type and the hexagonal B₄ type modifications of the pink sulphide have more or less the same susceptibility value whereas the green modification gives a much higher χ -value. The cause of these differences is discussed. Measurement of susceptibilities of the different preparations of MnS at different temperatures indicate that the Curie-Weiss law is applicable in their case.

It is generally recognised that manganous sulphide exists in two forms: rose and green. The grey variety reported in earlier literature has been shown to consist of the rose and the green varieties in varying proportions. The two forms are identical in composition (Antony and Donnini, *Gazzetta*, 1893, **23**, 560) but the difference in colour, density and particle size of the two preparations is so marked that they are usually assumed to represent two isomeric forms. As numerous cases are known where differences in colour, density, etc., are due to differences in physical state of the same substance, the question arises whether the transformation from rose to green manganous sulphide is due to a change in physical character or in molecular structure.

The magnetic method has recently afforded useful information on the structure of particularly the paramagnetic compounds. In general, if the electronic orbits of an atom are so disposed that their resultant magnetic moment is zero, the atom exhibits diamagnetism, whereas paramagnetic behaviour is the consequence of a resultant magnetic moment. Van Vleck ("Theory of Electric and Magnetic Susceptibilities", Oxford, particularly Chapter XI, p. 73), Sommerfeld ("Atombau," 4th Ed. p. 639), Bose (*Z. Physik*, 1927, **43**, 864) and Stoner (*Phil. Mag.*, 1929, **8**, 250) have obtained the following derivation:

$$\mu_B = \sqrt{4s(s+1) + l(l+1)}$$

which reduces to

$$\mu_B = \sqrt{4s(s+1)}$$

where l , the orbital moment, is fully quenched.

The paramagnetic salts of the transition metals give magnetic values which are, more or less, in accord with the theoretical values deduced on Van Vleck's formula for spin. The sulphides, however, have not been investigated so completely and systematically from the magnetic standpoint. There are often considerable differences in values found by different workers for the susceptibility of a particular sulphide and there are few precise determinations of the change of χ with temperature. Thus according to Wedekind (*Z. angew. Chem.*, 1924, **37**, 87) manganous sulphide has a susceptibility value of 44.32×10^{-6} but according to Wistrand ("Magnetiska susceptibiliteten hos kvarts, Tellur och Nagra Halnium fereringara", Upsala, 1916) this value is 64.8×10^{-6} . Haraldsen and Klein (*Z. anorg. Chem.*, 1934, **220**, 183) recently investigated the influence of temperature on its susceptibility and concluded that the susceptibility value approaches that calculated for manganous ion as the temperature is raised. Work on similar lines has been done by Mehmed and Haraldsen (*ibid.*, 1938, **235**, 193).

The observed susceptibility values of MnS are widely different from the theoretical values calculated on the Bose-Stoner formula for spin. It may be that these deviations are due to wide divergence from the Curie law,

$\chi_M = \frac{C_M}{T}$ and the magnetic behaviour of MnS is better represented by its

Weiss modification, $\chi_M = \frac{C_M}{T - \theta}$, since the divergence arises primarily from distortions produced by interatomic forces, etc., and these are taken into consideration in the Weiss law by the introduction of θ . The Bohr magneton number can be evaluated from the relationship,

$$\mu_B = 2.839 \sqrt{\chi_M(T - \theta)}$$

The present work was undertaken in order to study the magnetic behaviour of sulphides at different temperatures and to determine, if possible, whether the transformation from the rose to the green modification involves, a physical change or a molecular rearrangement.

EXPERIMENTAL.

The sulphide was prepared in two colours rose and green.

Rose Sulphides.

(1) Equal volume of $M\text{-MnCl}_2$ and $1.35M\text{-NH}_4\text{HS}$ were mixed together. The rose-coloured manganous sulphide so formed was washed with water

containing a little H_2S until free of chloride and sulphide. It was then washed six times with 20 c.c. portions of alcohol, twice with carbon disulphide and finally 3 more times with alcohol. The product was dried at 60° in an atmosphere of dry and purified hydrogen sulphide (Weiser and Milligan, *J. Phys. Chem.*, 1931, **35**, 2330).

(II) Equimolecular quantities of $M\text{-MnCl}_2$ and $2M$ -sodium sulphide were mixed. The product was washed and dried as described above.

Green Sulphides.

(I) Purified and dried H_2S was passed over the rose variety at 320° . The green sulphide so formed was washed with carbon disulphide to free it of any free sulphur and dried at 60° (Antony and Donnini, *loc. cit.*).

(II) Hydrogen sulphide was passed over manganous sulphate heated to 600° . The green sulphide so formed was washed with carbon disulphide to free it of sulphur, if any, and dried.

(III) 20 C.c. of $M\text{-MnCl}_2$, 25 c.c. of 11.6 $M\text{-NH}_4\text{OH}$, 30 c.c. of 1.35 $M\text{-NH}_4\text{HS}$ and 45 c.c. of water were mixed together. The green product began developing in a few minutes. It was left in contact with the solution for a few days when whole of the product turned green. It was washed and dried (Weiser and Milligan, *loc. cit.*).

Analysis.—Manganese was estimated by the pyrophosphate method. Sulphide was oxidised to sulphate with bromine and nitric acid, the sulphate precipitated as BaSO_4 , washed, ignited and weighed. The results of these analyses are given in Table I which also records the densities of the samples.

TABLE I.

Reading	Modification of MnS .	Density at 33° .	M a n g a n e s e		S u l p h u r	
			Expt.	Theor.	Expt.	Theor.
1	Rose I	3.246	63.12%	63.22%	36.79%	36.78%
2	„ II	3.250	63.16	„	36.73	„
3	Green I	3.970	63.22	„	36.81	„
4	„ II	3.965	63.18	„	36.77	„
5	„ III	4.012	63.20	„	36.75	„

Magnetic Data.

The magnetic susceptibility value of the samples was determined on a modified form of Gouy's magnetic balance.

For measurements at higher temperatures an electrically heated silica tube-furnace was used. The variations in temperature near the pole pieces on account of the furnace were not found to affect the field strength, because the pull per gram observed for a standard diamagnetic at various temperatures was found to be unaffected. In order to see whether the substance in the tube had actually attained the temperature recorded by the thermometer in the electric furnace, a magnetic examination of manganese pyrophosphate, prepared from analytically pure manganese sulphate, was undertaken. The calculation of temperature was made by the aid of the Curie-Weiss law, $\chi_M = \frac{C_M}{T - \theta}$, the value of $\theta (-23^\circ)$ being known for this compound. The observed and the calculated temperatures are tabulated below.

TABLE II.

Reading.	$\chi \times 10^6$.	Temperature °K.	
		Obs.	Calc.
1	101.73	305	—
2	95.80	325	325.3
3	88.20	355	355.3
4	76.30	414	414.3
5	68.70	463	462.7

The agreement between the two values is excellent.

The magnetic results are recorded in Table III. The value of μ_B is calculated from the formula

$$\mu_B = 2.839 \sqrt{\chi_M (T - \theta)}$$

where χ_M corresponding to the observed χ is calculated for one metallic atom per molecule and θ , the Curie point, is determined by plotting $1/\chi$, T graph and reading off θ as the intercept on the T -axis.

TABLE III.

Preparation.

ROSE I.

Reading.	Temp.	$\chi \times 10^6$.	$\chi_M \times 10^6$.	θ .	Mean		
					C_M .	C_M .	μ_B .
1	307°K	41.95	3650		4.478		
2	336	40.97	3564		4.477		
3	367	40.02	3482		4.482		
				-920°K		4.488	6.02
4	410	38.72	3369		4.481		
5	460	37.36	3250		4.485		
6	510	36.37	3164		4.525		

ROSE II

1	307	41.80	3637		4.389		
2	338	40.80	3550		4.394		
3	370	39.80	3463		4.398		
				-900°K		4.394	5.95
4	417	38.45	3335		4.391		
5	480	36.71	3194		4.409		
6	518	35.50	3089		4.381		

GREEN I.

1	307	64.15	5581		4.487		
2	337	61.80	5377		4.484		
3	367	59.63	5188		4.482		
				-497°K		4.475	6.01
4	417	56.30	4898		4.477		
5	477	52.78	4592		4.472		
6	510	51.04	4440		4.472		

TABLE III (*contd.*).

GREEN II.						
Reading.	Temp.	$\chi \times 10^6$.	$\chi_M \times 10^6$.	θ .	C_M .	Mean C_M μ_B .
1	307°K	64.20	5585		4.462	
2	337	61.90	5385		4.465	
3	365	59.86	5208		4.464	
				-492°K		4.462 6.00
4	411	56.50	4916		4.469	
5	483	52.62	4578		4.464	
6	507	51.20	4454		4.450	
GREEN III.						
1	307	64.10	5577		4.462	
2	337	61.70	5368		4.455	
3	366	59.51	5177		4.447	
				-493°K		4.448 5.99
4	417	56.21	4890		4.450	
5	483	52.30	4550		4.441	
6	510	50.81	4420		4.434	

DISCUSSION.

Prominent points which the foregoing investigation brings forth are .

(i) That the samples of the rose sulphide have a susceptibility value of $41.85 (\pm 0.10) \times 10^{-6}$ at 34°C. The $(1/\chi, T)$ graph gives a θ value of $-910^\circ \pm 10^\circ$ and the magneton number calculated on the Curie-Weiss formula works out to 5.95-6.02 which is in good accord with the theoretical value of 5.92, found for bivalent manganese on Van Vleck's formula for spin.

(ii) That the samples of the green sulphide prepared (1) by precipitation method, (2) by passing hydrogen sulphide over the rose sulphide and (3) by passing H_2S over manganous sulphate, gave χ value of $64.15 (\pm 0.05) \times 10^{-6}$. The Curie temperature was found to be -495 ± 3 . The magnetic moment is 5.99-6.01 which again is in excellent agreement with the calculated value.

The two varieties exhibit varying shades of colour. The green modification exhibits light green to dark green shades and yet it is remarkable that the different samples give practically the same susceptibility value which

suggests that the varying colour shades are due to differences in particle size which has been shown by Bhatnagar and co-workers (Bhatnagar, Verma and Haq, *Kolloid Z.*, 1937, **78**, 9 ; Lessheim, *Current Sci*, 1936, **6**, 119) to have little effect on the susceptibility. In this connection, it is of interest to note that Weiser and Milligan (*loc. cit*) arrived at a similar conclusion from X-ray analysis of the different preparations of green MnS. They found the X-radio-grams of the different specimens to be identical. The crystals had NaCl-type lattice and the value of a_0 for the crystals (whether light or dark green) was found to be 5.20\AA . Hence they concluded that the differences in shade do not involve changes in crystal structure but are dependent on difference in particle size.

Schnaase (*Z. physikal. Chem.*, 1933, **B20**, 89) recently carried out an X-ray examination of manganous sulphides and concluded that the sulphide exists in three modifications. The green sulphide has NaCl-lattice structure and the rose sulphide is, in general, a mixture of two modifications one of which has the zinc blende lattice (cubic B₃ type) and the other one the Wurtzit lattice (hexagonal B₄ type). Since the preparations (I) and (II) of the rose sulphide have been obtained by different chemical methods, they may be expected to contain the two modifications in different proportions and if susceptibility were influenced by the crystal structure, then the hexagonal type ought to have a different susceptibility from the cubic B₃ type with the result that the two preparations will give different χ values. The values for the two samples, however, are more or less the same. It, therefore, appears that change in crystal structure cannot be responsible, except perhaps to a very small extent, for the divergence noticed between the χ values of the rose and the green sulphides. The cause of this divergence in colour and susceptibility is rather to be sought in possible differences in the type of linkage involved in the two cases, but as the data on the band spectroscopy of manganese compounds are scanty, it is not possible to say how exactly the linkages differ in the two cases.

Incidentally it may be mentioned here that the density of the sulphides calculated from the X-ray analysis of Schnaase are 4.056, 3.289 and 3.780 respectively for the green, red (cubic) and red (hexagonal) modifications and these values are in fairly good accord with the experimental values recorded in Table I, which is indicative of the correctness of the X-ray data of Schnaase.

As regards the theoretical interpretation of θ , it is quite clear that it is not an atomic property but is due primarily to distortions produced by inter-atomic forces arising partly from the Heisenberg's exchange effect (Van Vleck, "Theory of Electric and Magnetic Susceptibilities", Oxford,

Chapter XII) and partly from the interaction of the orbital angular momentum. The Mn^{++} ion, as in MnS , is in the S-state and consequently devoid of any orbital angular momentum. Hence in this case θ value should be dependent entirely on Heisenberg exchange effect. This is actually shown to be the case with hydrated sulphates where the effect of exchange forces vanish on account of high "magnetic dilution" and as expected θ has zero value. The sulphides of transition elements, however, have very high "magnetic concentration" compared to the salts composed of a variety of atoms and it is, therefore, not surprising that the magnetic susceptibility of MnS deviates widely from the Curie law. It is also possible that some other disturbing influence, *e.g.*, crystalline field effects, etc., may be operating at the same time. It is, however, not possible to obtain a quantitative interpretation of θ on the concept of intrinsic molecular fields or distortions arising from interatomic forces. Nevertheless, the importance of this determination in explaining the anomalous magnetic behaviour of sulphides is clearly brought out.

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Received July 7, 1939.

DECOMPOSITION OF HYDROGEN PEROXIDE BY POTASSIUM FERROCYANIDE PART I.

BY BIJAN BIHARI LAL.

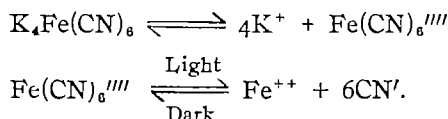
When a solution of $K_4Fe(CN)_6$ is illuminated by bright sunlight, a photochemical equilibrium is set up with the production of potassium aquopentacyanoferrite upto a maximum concentration. This equilibrium has been found to be reversible in the dark. The potassium aquopentacyanoferrite so produced is responsible for the phenomenon of after-effect observed in the decomposition of H_2O_2 by illuminated $K_4Fe(CN)_6$ solution. The aquo-salt is more reactive in the presence of excess of $K_4Fe(CN)_6$ than without it. The greater the time of exposure of $K_4Fe(CN)_6-H_2O_2$ mixture the greater is the velocity of decomposition of H_2O_2 whereas a solution of $K_4Fe(CN)_6$ illuminated to sunlight prior to its mixing with H_2O_2 in the dark, decomposes H_2O_2 with the same velocity irrespective of the duration of exposure. Based on these observations a mechanism of this reaction has been outlined.

Kistiakowsky (*Z. physikal. Chem.*, 1900, **36**, 431) observed that a mixture of H_2O_2 and $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$, or H_2O_2 and $K_4Fe(CN)_6$ exposed to light and then brought back to darkness decomposes with a much higher velocity than a reaction mixture which has not been illuminated. The insolation of the mixture, however short it might be, had the pronounced effect in that the activity continued long after illumination.

A detailed study of the literature on the subject reveals much confusion as regards the cause of enhanced reactivity of the illuminated mixtures of $K_4Fe(CN)_6$ and H_2O_2 . Kistiakowsky (*loc. cit.*) attributes this marked reactivity to the formation of a catalyser (possibly of a colloidal nature) which continues its active influence after subsequent darkening. Weigert (*Ann. Phys.*, 1907, *iv*, **24**, 243) suggests that insolation produces reaction nuclei which act catalytically. Winther (*Danske. Vidensk. Medd.*, 1920, **2**, No. 1, 1) explains the phenomenon by assuming gradual formation of an extremely stable substance, which catalytically accelerates the decomposition of H_2O_2 . Rao and Srikantan (*J. Indian Chem. Soc.*, 1933, **10**, 29), on the other hand, find this reaction to be very complicated and not unimolecular with respect to H_2O_2 , as observed by Kistiakowsky. Amanuel (*Z. Chem. Ind.-Kolloide*, 1911, **8**, 14) attributes enhanced reactivity in this reaction after illumination to the production of a photo-phase by insolation of $K_4Fe(CN)_6$ solution. From the above it is clear that inspite of so many theories, there is hardly any experimental evidence in their support.

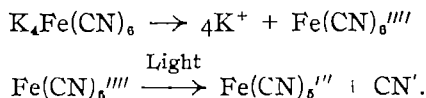
In order to elucidate the reaction mechanism, a knowledge of the properties of aqueous solution of $K_4Fe(CN)_6$ is necessary, but there is a

difference of opinion on this question. According to Matuschek (*Chem. Z.*, 1901, **25**, 565) ferric hydroxide is produced by insolating $K_4Fe(CN)_6$ solution. Haber (*Z. Elektrochem.*, 1905, **10**, 846) and Foster (*J. Chem. Soc.*, 1906, **89**, 916) suggest the following scheme:—



Baudisch (*Ber.*, 1922, **55**, 2701), on the other hand, finds that ferric hydroxide, HCN and OH ions are produced on exposing $K_4Fe(CN)_6$ solution to sunlight. Baur (*Helv. Chim. Acta*, 1925, **8**, 403) observes that ferrocyanide decomposes into ferric hydroxide sol, the hydroxide being later precipitated. This is due to ferricyanide impurity always present in ferrocyanide, which is itself photochemically insensitive. Thus the above authors are inclined to believe that either one, or more of the products Fe^{++} , Fe^{+++} , and ferric hydroxide sol results from the insolation of $K_4Fe(CN)_6$ solution.

Timori (*Z. anorg. Chem.*, 1927, **167**, 145), however, established by spectroscopic evidence that the absorption spectrum of illuminated solution of the salt is identical with that of potassium aquopentacyanoferrite, and proposed the following mechanism:—



He states that the light reaction is a reversible one.

In order to elucidate the reaction mechanism of illuminated $K_4Fe(CN)_6$ and H_2O_2 in the light of the above theories, it was thought best to add Fe^{++} , Fe^{+++} , and colloidal ferric hydroxide to a mixture of $K_4Fe(CN)_6$ and H_2O_2 in the dark and study the velocity constant. It was, however, found that colloidal ferric hydroxide, prepared by adding NaOH to ferric chloride and subsequently dialysing it for three months till $AgNO_3$ ceased to show any opalescence with the dialysate, did not accelerate the decomposition of H_2O_2 either in presence or absence of $K_4Fe(CN)_6$. Ferrous and ferric ions could not exist as both these ions should show blue colour with $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ generated by the oxidation of $K_4Fe(CN)_6$ by H_2O_2 . Moreover, reactions performed with $N/6-H_2O_2$ and 0.005% solution of ferric chloride (the same concentration as of sodium aquopentacyanoferrite) did not give an appreciable velocity of decomposition of H_2O_2 , both in presence and absence of $K_4Fe(CN)_6$. Ferric ion is thus proved to be of no reactivity so far as this reaction is concerned, at least at the concentration

stated above, and at this concentration $\text{Na}_3\text{Fe}(\text{CN})_6 \cdot \text{H}_2\text{O}$ with $\text{K}_4\text{Fe}(\text{CN})_6$ produces considerable activity as shown in the following experiments. Having eliminated all the above possibilities, it was considered desirable to test the hypothesis advanced by Iimori (*loc. cit.*) that $\text{K}_4\text{Fe}(\text{CN})_6$ on illumination is converted into potassium aquopentacyanoferrite.

EXPERIMENTAL.

For the preparation of sodium aquopentacyanoferrite (as the potassium salt is not easily available) a method proposed by Hofmann (*Annalen*, 1900, **312**, 31) was adopted. Sodium nitroprusside (20 g.) was treated with 10 g. of anhydrous sodium carbonate dissolved in 80 c.c. of water. To this was added 7 g. of hydroxylamine hydrate hydrochloride, dissolved in a little water, cooled with ice. Evolution of gas began immediately and the solution was coloured greenish brown. After one hour the sodium aquo-salt was precipitated with three times the volume of alcohol as a brown gummy mass. By repeated dissolving in water and precipitation with methyl alcohol, the substance was finally obtained as a lemon-yellow powder. The temperature was kept below 5° . The preparation was dried over sulphuric acid at 17 mm. pressure for 48 hours, when yellowish brown aquo-salt of the formula* $\text{Na}_3\text{Fe}(\text{CN})_6 \cdot \text{H}_2\text{O} + \text{H}_2\text{O}$ was obtained.

It is clear from the above that if potassium aquopentacyanoferrite is produced by insolation of aqueous solution of $\text{K}_4\text{Fe}(\text{CN})_6$, the former substance should decompose hydrogen peroxide in the dark. In the following experiments, Kahlbaum's pure $\text{K}_4\text{Fe}(\text{CN})_6$ was used in conductivity water. Pure H_2O_2 was prepared by taking Kahlbaum's pure Na_2O_2 and pure H_2SO_4 and distilling the hydrogen peroxide at 17 mm. pressure in Jena glass apparatus. The distillate gave test for traces of H_2SO_4 , which was possibly mechanically carried over. Hence the distillate was shaken with pure CaCO_3 , filtered and redistilled. In this way pure aqueous H_2O_2 was obtained which was free from chloride and sulphate.

Though the velocity constants obtained here leave something to be desired, they are as good as those reported in the literature (Kistiakowsky, *loc. cit.*, Bredig, *Z. physikal. Chem.*, 1899, **31**, 258; 1901, **37**, 3, 323; Brossa, *ibid.*, 1909, **66**, 161; Tartar and Schaffer, *J. Amer. Chem. Soc.*, 1928, **50**, 2604). It is necessary to mention here that this reaction is highly

* The iron content was gravimetrically estimated and a variation of +0.9% from the theoretical value was obtained. This may be due to a slight impurity and also to the highly hygroscopic nature of the compound.

susceptible to traces of impurities, for in parallel experiments sometimes there occur errors of more than 20% which cannot be attributed to the errors in manipulation, but can only be accounted for by the impurities in the air of the laboratory. The surface of the reaction vessel also has a disturbing effect. The reactions were carried in the dark room in duly cleaned and steamed Jena glass-stoppered bottles, and 5 c.c. of the reaction mixture were taken out after definite time intervals. $N/20.96\text{-KMnO}_4$ was used to determine the change in concentration of H_2O_2 . The amount of KMnO_4 taken up by $\text{K}_4\text{Fe}(\text{CN})_6$ was subtracted from the total amount of KMnO_4 used. The strengths of H_2O_2 and $\text{K}_4\text{Fe}(\text{CN})_6$ were $N/6$ and $M/64.2$ respectively. The total volume of the reaction mixture in all the experiments was 50 c.c. $\text{K}_4\text{Fe}(\text{CN})_6$ and sodium aquopentacyanoferrite solutions were prepared in the dark and kept in black bottles. The reactions were carried out at $35 \pm 0.1^\circ$ in the dark room and unilluminated solutions were always used unless otherwise stated. The reaction is unimolecular with respect to H_2O_2 and therefore

$$K = \frac{1}{t} \log \frac{a}{a-x}.$$

The amount of KMnO_4 taken up by the ferrite is negligibly small

In the following tables, allowance made for $\text{K}_4\text{Fe}(\text{CN})_6$ in 5 c.c. of reaction mixture = 1.6 c.c. of $N/20.96\text{-KMnO}_4$.

TABLE I.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 25 c.c. of $M/32.10\text{-K}_4\text{Fe}(\text{CN})_6$ (0.3242 g.).

Time.	KMnO_4 .	Corr. vol.	$K \times 10^5$.	Time.	KMnO_4 .	Corr. vol.	$K \times 10^5$.
0 min.	17.50 c.c.	15.90	—	261 min.	11.45 c.c.	9.85	80
26	16.80	15.20	76	295	10.60	9.00	84
102	15.00	13.40	73	336	9.30	7.70	94
150	13.95	12.35	73				

TABLE II.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 25 c.c. of $\text{Na}_3\text{Fe}(\text{CN})_6 \cdot \text{H}_2\text{O}$ (0.0025 g.).

Time.	KMnO_4 .	$K \times 10^5$.	Time.	KMnO_4 .	$K \times 10^5$.
0 min.	15.90 c.c.	—	111 min.	13.35 c.c.	68
5	15.45	250	160	12.70	61
35	14.50	114	240	11.55	58
61	14.15	83	305	10.85	54

TABLE III.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 10 c.c. of $M/25\ 68\text{-K}_4\text{Fe(CN)}_6$ (0.3242 g.) + 10 c.c. of $\text{Na}_3\text{Fe(CN)}_5\text{H}_2\text{O}$ (0.0025 g.) + 5 c.c. of water. $\text{K}_4\text{Fe(CN)}_6$ was added to H_2O_2 and to this mixture was added the aquo-salt.

Time	KMnO ₄	Corr. vol	$K \times 10^5$.	Time	KMnO ₄	Corr. vol.	$K \times 10^5$.
0 min.	17.50 c.c.	15.90	—	57.5 min	3.50 c.c.	1.90	1604
26	7.35	5.75	1699	66	3.05	1.45	1576
34.5	5.90	4.30	1646	81	2.45	0.85	1570
46	4.60	3.00	1575	102	1.95	0.35	1624

TABLE IV.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 10 c.c. of $M/25\ 68\text{-K}_4\text{Fe(CN)}_6$ (0.3242 g.) + 2 c.c. of $\text{Na}_3\text{Fe(CN)}_5\text{H}_2\text{O}$ (0.0005 g.) + 13 c.c. of water.

Time.	KMnO ₄ .	Corr. vol.	$K \times 10^5$.	Time.	KMnO ₄ .	Corr. vol.	$K \times 10^5$.
0 min	17.50 c.c.	15.90	—	68 min.	6.45 c.c.	4.85	758
24.5	11.75	10.15	796	79	5.45	3.85	780
43	9.15	7.55	752	102	4.10	2.50	788
55	7.80	6.20	744	125	3.15	1.55	809

TABLE V.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 10 c.c. of $M/25\ 68\text{-K}_4\text{Fe(CN)}_6$ (0.3242 g.) + 0.40 c.c. of $\text{Na}_3\text{Fe(CN)}_5\text{H}_2\text{O}$ (0.0001 g.) + 14.6 c.c. of water.

Time.	KMnO ₄ .	Corr. vol	$K \times 10^5$.	Time.	KMnO ₄	Corr. vol.	$K \times 10^5$.
0 min	17.50 c.c.	15.90	—	102 min.	10.25 c.c.	8.65	259
27	14.80	13.20	299	125	9.30	7.70	252
41.5	13.55	11.95	299	253	5.30	3.70	250
60	12.50	10.90	273	284	4.50	2.90	260
78	11.60	10.00	258				

TABLE VI.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 25 c.c. of $M/32\cdot10\text{-K}_4\text{Fe(CN)}_6$ (0.3242 g.).

A.				B. Exposed to bright sunlight for 2 min. and then at once mixed with H_2O_2 in the dark.			
Time.	KMnO ₄	Corr. vol.	$K \times 10^5$	Time.	KMnO ₄	Corr. vol.	$K \times 10^5$
0 min.	16.70 c.c.	15.10	—	0 min	16.55 c.c.	14.95	—
45	15.60	14.00	73	4.5	14.65	13.05	1316
79	14.40	12.80	91	24	9.65	8.05	1126
120	13.30	11.70	92	40	6.65	5.05	1178
187	11.25	9.65	104	50	5.35	3.75	1201
220	10.05	8.45	115	61	4.35	2.75	1206
				74	3.35	1.75	1259
				86	2.70	1.10	1317

TABLE VII.

25 C.c. of $N/3\text{-H}_2\text{O}_2$ + 25 c.c. of $M/32\cdot10\text{-K}_4\text{Fe(CN)}_6$ (0.3242 g.).

A. Exposed to bright sunlight in quartz tube for 5 min. then mixed with H_2O_2 at once in the dark room.				B. Exposed in quartz tube to bright sunlight for 5 min. but used after keeping in the dark room for 20 min.			
Time.	KMnO ₄	Corr. vol.	$K \times 10^5$	Time.	KMnO ₄	Corr. vol.	$K \times 10^5$
0 min.	16.20 c.c.	14.60	—	0 min	16.50 c.c.	14.90	—
12	11.55	9.95	1388	27	16.20	14.60	33
25	8.60	7.00	1277	54	15.40	13.80	62
38	6.45	4.85	1260	180	10.85	9.25	115
50	4.90	3.30	1292	210	10.00	8.40	119
66	4.00	2.40	1188	240	9.20	7.60	122
95	2.85	1.25	1124	270	8.00	6.40	136
				300	7.50	5.90	134

In the control the value of $K \times 10^5$ is 83.

TABLE VIII.

25 C.°c. of $N/3 \cdot H_2O_2$ + 25 c. c. of $M/32 \cdot 10 \cdot K_4Fe(CN)_6$ (0.3242 g.).

A.				B.			
Time.	KMnO ₄ .	Corr. vol.	$K \times 10^5$.	Time.	KMnO ₄	Corr. vol.	$K \times 10^5$
0 min.	17.00 c.c.	15.40	—	0 min.	17.05 c.c.	15.45	—
50	15.70	14.10	76	50	15.65	14.05	83
70	9.20	7.60	—	85	4.35	2.70	—
90	5.70	4.10	1340	90	3.60	2.00	2766
106.5	4.10	2.50	1323	100	2.60	1.00	2733
120	3.25	1.65	1326				
150	2.20	0.60	1380				

N.B.—After 50 min. the reaction mixtures in Tables A and B were exposed to sunlight for 1 and 5 minutes respectively, and then they were removed to the dark room and the velocity constants measured.

DISCUSSION.

The above data (Tables I, II, III) show that sodium aquopentacyanoferrite enhances the rate of decomposition of H_2O_2 in the dark considerably only when excess of $K_4Fe(CN)_6$ is present in the reaction mixture. If the aquo-salt alone is used in the dark without the addition of $K_4Fe(CN)_6$, the rate of decomposition is much lower compared to the above reaction velocity (*cf.* Tables II and III).

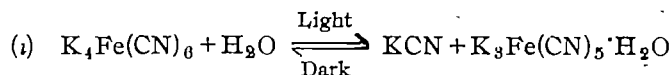
Another important fact brought out from the data in Tables VI and VII is that the time of insolation of aqueous solution of $K_4Fe(CN)_6$, prior to its addition to H_2O_2 in the dark, does not have any effect on the final reaction rate. In other words, insolation of $K_4Fe(CN)_6$ for any length of time (2 min. or 5 min.) produces the active substance, potassium aquopentacyanoferrite, upto a maximum concentration and further exposure does not raise its concentration.

Further analysis of the above experimental results revealed the very important fact that if a solution of $K_4Fe(CN)_6$ alone is exposed to sunlight and immediately used for the decomposition of H_2O_2 in the dark, the

velocity constant is very much higher and compares well with that obtained with exposed mixture of $K_4Fe(CN)_6$ and H_2O_2 . If, on the other hand, an illuminated solution of $K_4Fe(CN)_6$ is kept in the dark for 10-20 minutes before using, it does not show any increase in the velocity when compared with blanks. But, on the contrary, the initial velocity constants are definitely lower than those obtained in controls, and towards the end of the reaction, the values of the velocity constants approach the values obtained in controls (cf. Tables VI and VII).

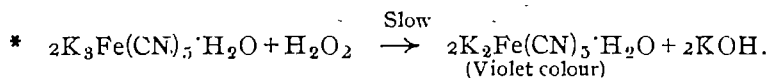
It is also clear from the data available (Tables III, IV, V) that the greater the amount of sodium aquopentacyanoferrite taken with the same amount of $K_4Fe(CN)_6$, the greater is the value of K .

The following mechanisms may be suggested on the evidence adduced from the foregoing experiments:—

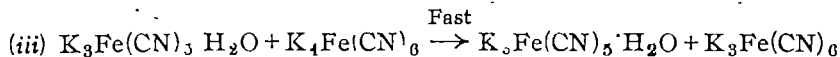


i.e., a photochemical equilibrium is established and the reaction is reversible in the dark.

(ii) $K_3Fe(CN)_5 \cdot H_2O$ so produced vigorously decomposes H_2O_2 , as is evident from the violent evolution of oxygen from the mixture. At the same time oxidation of $K_3Fe(CN)_5 \cdot H_2O$ takes place



Potassium aquopentacyanoferrate is produced, which is further reduced as follows:—



In the light of the above mechanism the above mentioned facts are readily explained.

* In an illuminated mixture of $K_4Fe(CN)_6$ and H_2O_2 , traces of aquopentacyanoferrite also exist and four reactions actually proceed in such a mixture in the dark, resulting in the disappearance of H_2O_2

(i) Decomposition of H_2O_2 by aquopentacyanoferrite generated by illumination of $K_4Fe(CN)_6$.

(ii) Decomposition of H_2O_2 by $K_4Fe(CN)_6$ [The dark reaction which was originally going on in the mixture of H_2O_2 and $K_4Fe(CN)_6$ before illumination].

(iii) Reduction of H_2O_2 by aquoferrite.

(iv) Reduction of H_2O_2 by $\text{K}_4\text{Fe}(\text{CN})_6$.

Since the evolution of oxygen from an illuminated mixture of H_2O_2 and $\text{K}_4\text{Fe}(\text{CN})_6$ is very vigorous, it is evident that the fast disappearance of H_2O_2 is mainly due to its decomposition and not due to its reduction. Some H_2O_2 is no doubt used up in oxidising the ferrite to ferrate which imparts a violet colour to the reaction mixture not containing $\text{K}_4\text{Fe}(\text{CN})_6$ (cf. Table II). H_2O_2 also oxidises a part of $\text{K}_4\text{Fe}(\text{CN})_6$ to $\text{K}_3\text{Fe}(\text{CN})_6$. The velocity of decomposition of H_2O_2 by $\text{K}_4\text{Fe}(\text{CN})_6$ in the dark (which includes the reactions (ii) and (iv)) is very small compared to the velocity of decomposition of H_2O_2 by illuminated $\text{K}_4\text{Fe}(\text{CN})_6$; only a small portion of the total H_2O_2 is so decomposed. The aquo-salt being in very minute amounts, only a very small fraction of the total H_2O_2 present suffers reduction and the rest undergoes rapid catalytic decomposition. During a certain time interval, therefore, the amount of H_2O_2 decomposed by the ferrite is very much more in comparison to the amount of H_2O_2 lost in the reactions (ii), (iii) and (iv). Thus the disturbing effect of the reactions (ii), (iii) and (iv) is small and the values of K are fairly constant, when calculated on the basis of the unimolecular reaction. It is notable that in mixtures of H_2O_2 and aquoferrite, the value of K is not constant, but falls rapidly (cf. Table II) because $\text{K}_4\text{Fe}(\text{CN})_6$ is not there to maintain the concentration of the aquoferrite.

When the aquopentacyanoferrite is used with H_2O_2 without the addition of $\text{K}_4\text{Fe}(\text{CN})_6$, decomposition of H_2O_2 takes place as is evident from the evolution of oxygen, and aquopentacyanoferrate of violet colour is also produced due to oxidation as shown in equation (ii). The violet colour of aquoferrate as described by Hofmann (*loc. cit.*) is actually obtained in the experiment described in Table II. The aquopentacyanoferrite decomposes H_2O_2 very quickly and the ferrate, which is produced due to oxidation, slowly decomposes H_2O_2 towards the latter part of reaction (cf. Table II). Thus the change of aquopentacyanoferrite to aquopentacyanoferrate also accounts for a higher value of K in the beginning, and a slower rate towards the later stage, which is due to the slow decomposition of H_2O_2 by the ferrate.

On the other hand, a mixture of H_2O_2 with $\text{K}_4\text{Fe}(\text{CN})_6$ and a solution of sodium aquopentacyanoferrite gives a very high rate of decomposition, which is maintained at its initial value to the very end of the reaction (Tables III, IV, V). This is explained by the proposed mechanism con-

sidering the equations (ii) and (iii). The aquoferrate once produced reacts immediately with $K_4Fe(CN)_6$ and reforms aquoferrite, so that to all intents and purposes, the initial amount of aquoferrite added is always present as such as long as excess of potassium ferrocyanide is there, and this accounts for a uniformly high reaction rate. Also the end-solution is yellow and not violet, which definitely points out that ferrate is not present. The reaction in equation (iii) is very fast compared to the reaction in equation (ii).

It is clear from Table VIII that the greater the time of exposure of $K_4Fe(CN)_6-H_2O_2$ mixture, the greater is the resultant velocity constant, which fact is also in conformity with the suggested reaction mechanism. Since excess of $K_4Fe(CN)_6$ is always present in the mixture, potassium aquopentacyanoferrite produced by insolation reacts with H_2O_2 disturbing the equilibrium (i). More of the aquoferrite is, therefore, produced again to maintain the equilibrium and hence on the whole, a greater amount of aquoferrite is produced than would have resulted for the same duration of insolation, if H_2O_2 were not present to remove it by oxidation from the field of reaction. This increased concentration of the aquoferrite increases the decomposition rate considerably according to the data in Tables III, IV and V. As soon as the mixture is removed to the dark room, the reaction goes to the left so that ultimately a definite quantity of potassium aquopentacyanoferrite, depending upon the time of exposure, participates in the reaction. Hence the greater the time of exposure, the greater is the value of K .

That the time of exposure of $K_4Fe(CN)_6$ alone, prior to mixing with H_2O_2 in the dark, has no particular effect on the final result is also accounted for by the existence of an equilibrium as shown in equation (i). It has been seen (Tables III-V) that the greater the amount of sodium aquopentacyanoferrite used, the greater is the rate. But it is observed (Tables VI and VII) that whatever be the time of exposure (2 min. or 5 min.) the same rate is obtained when $K_4Fe(CN)_6$ is mixed with H_2O_2 in the dark after insolation. This suggests that by exposing a solution of $K_4Fe(CN)_6$ of a particular strength (in this case $M/32.10$) the same amount of aquoferrite is produced irrespective of the duration of exposure, otherwise the velocity constants should have been different. This can only be accounted for by the existence of an equilibrium as in (i). Also the above equilibrium is reversible in the dark, as shown by Table VII.

A proof in support of the above mechanism is found in the fact that the end-solution in all the above experiments containing illuminated $K_4Fe(CN)_6$ gives a greenish colouration with dilute HCl, proving thereby the presence

of aquoferrite. This proves that potassium aquopentacyanoferrite remains unchanged in the presence of excess of $K_4Fe(CN)_6$, whereas in the absence of the latter it is completely converted to ferrate. The violet colour of aquopentacyanoferrate is discharged by $K_4Fe(CN)_6$ with the result that the aquopentacyanoferrite of yellow colour is again produced with the simultaneous formation of $K_3Fe(CN)_6$ as shown in equation (iii).

CONCLUSION.

When a solution of $K_4Fe(CN)_6$ is illuminated by bright sunlight, a photochemical equilibrium is set up with the production of potassium aquopentacyanoferrite upto a maximum concentration. The latter decomposes H_2O_2 and the rate is maintained at the high value till the end of the reaction. But when a reaction mixture, $K_4Fe(CN)_6 + H_2O_2$ is exposed to sunlight, the aquo-salt produced is immediately removed from the field of reaction by H_2O_2 , and the photochemical equilibrium is shifted to the right with the additional production of aquo-salt which again reacts with H_2O_2 . On the whole, therefore, a greater concentration of the aquo-salt is available when a mixture of $K_4Fe(CN)_6$ and H_2O_2 is exposed to sunlight than when $K_4Fe(CN)_6$ alone is exposed and the greater concentration of the above substance is responsible for higher value of K .

The cause of the after-effect known in the decomposition of H_2O_2 by $K_4Fe(CN)_6$ after exposure has been found to be due to the reversible photoformation of potassium aquopentacyanoferrite which decomposes H_2O_2 in the dark with a uniformly high velocity, in the presence of excess of $K_4Fe(CN)_6$. The after-effect is not due to the formation of colloidal ferric hydroxide or ferric ion.

The aquopentacyanoferrite is more active in presence of $K_4Fe(CN)_6$ than without it, which is proved to be due to the oxidation of ferrite to ferrate by H_2O_2 , and the reduction of ferrate to ferrite by the excess of $K_4Fe(CN)_6$.

A solution of $K_4Fe(CN)_6$, exposed to sunlight either for 2 or 5 minutes gives the same value of K with H_2O_2 in the dark. This value is very much higher than that obtained in blanks.

The greater the time of exposure of the mixture, $K_4Fe(CN)_6-H_2O_2$, the greater is the value of the velocity constant.

A solution of $K_4Fe(CN)_6$ exposed to sunlight for 2 or 5 minutes, and then kept in the dark for 10-20 minutes, does not give the same high value of K ,

as is obtained with $K_4Fe(CN)_6$ solution exposed and at once used with H_2O_2 . Hence the production of aquoferrite from $K_4Fe(CN)_6$ by light is a reversible reaction in the dark.

A pre illuminated solution of $K_4Fe(CN)_6$ which has been kept in the dark for some minutes and then used gives a slightly lower value of K in the initial stages and it tends to rise in the end.

According to the second part of the suggested mechanism, the main cause of the fast disappearance of H_2O_2 is the decomposition and not its power of oxidation. This fact is corroborated by the violent evolution of oxygen during the reaction.

My best thanks are due to Prof. P. S. MacMahon for suggesting the problem and guiding me in the earlier part of the work and to Dr. A. C. Chatterji for guiding me in the latter part of the work. My thanks are also due to Lucknow University for the grant of a fellowship which enabled me to carry on this investigation.

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Received June 15, 1939.

ON THE BITTER PRINCIPLE FROM *ANDROGRAPHIS PANICULATA*, NEES. PART I.

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The formula and other properties ascribed to andrographolide, $C_{20}H_{30}O_5$, by Gorter have been confirmed. The presence of a methylenedioxy group has been proved. Triacetylandrographolide of Gorter could not be prepared. Hydrolysis of andrographolide gave two isomeric acids, $C_{20}H_{32}O_6$, one of which is probably identical with the known andrographolic acid. Iodine chloride indicated one double bond, but the catalytic hydrogenation of the bitter principle gave a dihydro derivative different from an isomeric dihydroandrographolide obtained by the action of stannous chloride.

Andrographis paniculata, Nees ("Kalmegh" in Bengali), is extensively used in the treatment of various diseases, such as disorders of the bowels and liver, colic, undiagnosed fevers, and also as a cholagogue and anthelmintic.

Dymock (*Pharmacographia Indica*, Vol. III, p. 47) reports the presence of a non-alkaloidal bitter principle, while Abderhalden ("Biochem. Handlexikon, Vol. VII, p. 230) mentions the bitter principle, andrographolide $C_{18}H_{27}O_4$, as colourless, odourless plates, sparingly soluble in water, but more easily in chloroform and ethyl acetate. It does not give tests for a glucoside, but dissolves in concentrated sulphuric acid with a yellow colour which changes to purple-red on warming (Boorsma, *Jahresber. Pharm.*, 1897, 32, 43). Bhaduri (*Amér. J. Pharm.*, 1914, 84, 389) described two bitter principles (of *A. paniculata*), obviously impure, one of which formed yellow crystals, m.p. 206° , and contained OH groups, while the other was amorphous, m.p. 185° , to which the improbable formula, $C_{18}H_{21}O_8$ or $C_{19}H_{22}O_8$ was given. Gorter (*Rec. trav. chim.*, 1911, 80, 151; 1914, 83, 236) prepared andrographolide in a pure condition. He assigned to it the formula, $C_{20}H_{30}O_5$ (m.p. 218° with decomposition; $[\alpha]_D^{26} = -126^\circ$ in 2% acetic acid). He also prepared a triacetyl derivative, m.p. 129° , and an amorphous additive product with hydrogen bromide namely $C_{20}H_{33}O_5Br_3$, and andrographolic acid, m.p. 188° (needles) having $[\alpha]_D^{26} = +14.4^\circ$. Gorter's conclusions are that andrographolide contains three OH groups one of which appears to be tertiary, besides a lactone ring and more than one double bond.

We have reinvestigated this bitter principle. Andrographolide, isolated by us from the leaves of fully mature plants in varying yields (1.5–2.5 per cent.) and purified by repeated crystallisations from suitable solvents, melts

at 220° (decomp.). It is extremely bitter even at very great dilutions. From analytical and saponification data the molecular formula, $C_{20}H_{30}O_5$, is confirmed. It has $[\alpha]_D^{20} = -123.5^\circ$ in 2% glacial acetic acid solution. It does not give tests for a glucoside and is indifferent towards ferric chloride, ammoniacal silver salts, Fehling's solution, bromine water and cold permanganate solution. Oxidation, however, takes place with warm permanganate solution. It is insoluble in cold dilute mineral acids or alkali, but dissolves in concentrated hydrochloric acid with a pink, and in concentrated sulphuric acid with a yellow colour, and is thrown down unchanged on dilution with water. The sulphuric acid solution becomes deep brown and shows a bright green fluorescence on warming.

On prolonged contact with or on warming with alkali, specially in presence of alcohol, andrographolide dissolves to a light yellow solution and is precipitated as a gum on acidification. If, however, the alkaline solution be carefully acidified, a crystalline acid, $C_{20}H_{32}O_6$, m.p. 156°, is obtained. We shall refer to this acid as *isoandrographolic acid*. It gives insoluble barium, calcium and lead salts and is transformed into the original substance on warming with dilute hydrochloric acid. On being warmed with a slight excess of ammonia, followed by acidification in the cold, *isoandrographolic acid* is converted into another isomeric acid, m.p. 180°, which is presumably identical with Gorter's andrographolic acid, $C_{20}H_{32}O_6$.

Andrographolide is recovered practically unchanged after heating for 5-10 minutes with acetic anhydride and fused sodium acetate. This is in sharp contrast with Gorter's experience. Acetylation in presence of a trace of concentrated sulphuric acid, however, rapidly gives a deep brown solution with a strong green fluorescence, from which a fluorescent gum is precipitated on dilution with water. This is presumably due to loss of water and carbon dioxide, since a similar product is obtained by dry heating at 225-30°, when the formation of water and carbon dioxide could be easily demonstrated. This indicates the presence of at least one OH group. The lactone ring probably supplies the carbon dioxide. Attempts to obtain a chloro compound by treating andrographolide with phosphorus oxychloride on the water-bath results in the formation of a solid product, which melts at 120° after purification and contains both phosphorus and chlorine. This is, however, found to be free from methylenedioxy group, which is originally present in andrographolide (*vide infra*). Treatment with phenyl isocyanate gives a white amorphous solid, m.p. 90-95°, containing nitrogen. All these reactions prove the presence of at least one OH group in the molecule. Distillation of andrographolide with 33% sulphuric acid gives formaldehyde. This indicates the presence of a methylenedioxy group.

All the oxygen atom in andrographolide are thus accounted for. Two form the lactone ring, two are present in the methylenedioxy group, while the fifth is present as an alcoholic group, probably of tertiary character.

Reduction of andrographolide with stannous chloride gives a dihydro derivative, m. p. 200° , whereas catalytic hydrogenation in presence of palladium gives an isomeric compound, m. p. 206° . A mixture of the two compounds melts at $183-85^{\circ}$, and hence they are different from each other. Bromination gives an additive compound, contaminated with higher bromination products. Andrographolide absorbs one molecule of hydrogen chloride, forming a compound, $C_{26}H_{31}O_5Cl$, m. p. $55-57^{\circ}$, but the additive compound with hydrogen bromide could not be obtained pure. Iodine chloride indicates the presence of one double bond. Andrographolide is consequently an unsaturated compound.

According to Gorter, andrographolide reacts with anhydrous formic acid forming a compound, m. p. 215° ; we find, however, that our compound remains unchanged by short treatment with this reagent.

EXPERIMENTAL.

Isolation of Andrographolide.—The leaves collected from fully mature plants were dried in the sun, powdered and then extracted with chloroform in a Soxhlet. In the course of two days much green crystalline material was found to float in the solvent, which had assumed a dark greenish brown colour. The green substance was filtered off and further amounts were obtained on concentration of the mother-liquor and cooling. No attempt was made to exhaust the leaves of the bitter principle as this invariably led to difficulty in purification. The crude green mass was washed with benzene, and re-extracted with chloroform in a smaller Soxhlet to remove much of the leaf-pigments. The crystals obtained from the chloroform extract were washed with benzene and repeatedly crystallised from methyl and ethyl alcohol (charcoal) till completely free from colouring matters. Andrographolide was finally obtained as perfectly colourless rectangular plates, or almost cubical crystals, m. p. 220° (decomp.). The yield varied unaccountably from lot to lot, depending possibly on the maturity of the plants. The best yield (2.5%) was obtained from mature plants grown in one of the authors' garden at Dacca, while the samples purchased locally gave as low as 0.8–1% andrographolide.

Andrographolide is sparingly soluble in water, petroleum ether, carbon disulphide, benzene and ether, but dissolves easily in hot chloroform, ethyl

and methyl alcohols, pyridine, ethyl acetate, acetic acid and phenol. (Found: C, 68.0; H, 8.41. $C_{20}H_{30}O_8$ requires C, 68.57; H, 8.57 per cent). A 2% solution in glacial acetic acid in 1 dm. tube showed a rotation of -2.47° . Hence $[\alpha]_D^{30} = -123.5^\circ$.

0.4208 G. of the substance was heated on the water-bath for 2 hours with 7 c.c. of baryta (1 c.c. $\equiv 4.175$ c.c. $N/10-H_2SO_4$) and the excess of alkali was titrated against $N/10-H_2SO_4$. The alkali used up was 3 c.c. Therefore, M.W., is 336, on the basis of a monobasic acid.

isoAndrographolic Acid.—Andrographolide (3.5 g.) was heated on the water-bath for 1 hour with 30 c.c. (7 g. KOH) of aqueous-alcoholic alkali when a clear pale yellow solution resulted. This was shaken for 5 minutes with animal charcoal, filtered, the filtrate cooled in ice and acidified with dilute hydrochloric acid till just acidic to Congo-red. The separated colourless needles were collected, washed with water and recrystallised from hot water. m.p. 156° . It liberates carbon dioxide vigorously from a bicarbonate solution. (Found: C, 64.99, 65.03; H, 8.81, 8.21. $C_{20}H_{32}O_8$ requires C, 65.2; H, 8.7 per cent). It gives insoluble barium, calcium, silver and lead salts. The crystalline barium salt was analysed [Found: Ba, 15.00 ($C_{20}H_{32}O_8$) $_2$ Ba requires Ba, 15.7 per cent].

The same acid was obtained when andrographolide (1 g.) was heated with 2.5 c.c. of baryta solution (2%), the lactone passing in solution within 15 minutes and then the barium salt crystallising out. When the filtered solution was cooled and neutralised with hydrochloric acid, *isoandrographolic acid* was precipitated. If, however, the entire product containing the barium salt be acidified with hydrochloric acid and warmed, the crystals of the barium salt dissolves and on cooling andrographolide is obtained, m.p. after purification, 220° , either alone or admixed with andrographolide.

Andrographolic Acid.—When *isoandrographolic acid* was warmed for 10 minutes with a slight excess of ammonia, cooled and acidified with hydrochloric acid, a little gummy matter was first thrown down. After its removal, the solution gradually deposited silky crystals of another acid, m.p. 180° .

Additive Compounds with Bromine and Halogen Acids.—(a) To andrographolide (3 g.), dissolved in concentrated hydrochloric acid (41.16, 30 c.c.), bromine (1 c.c.) was slowly added. There was rapid decolourisation with evolution of heat. After standing for 1 hour, the mixture was poured into ice. A yellow flocculent solid separated, which was washed with water, dried and purified by dissolving in methyl alcohol and pouring into much water. The pale yellow powder thus obtained melted indefinitely at $128^\circ-140^\circ$ (Found: Br, 38.28. $C_{20}H_{30}Br_2$ requires Br, 31.37 per cent).

(b) To 2 g. of andrographolide dissolved in acetic acid (30 c.c.) was added 1 c.c. of bromine in 10 c.c. of acetic acid. One such sample was kept in bright sunlight for 2 hours and the other was kept overnight. The contents of each were poured into a strong solution of ammonium acetate, when a flocculent precipitate was obtained. This was purified as described above. Identical products were obtained in the two cases, m.p. 110-12° (decomp). (Found: Br, 34.36, 34.24. $C_{20}H_{30}O_8Br_2$ requires Br, 31.37 per cent).

(c) Andrographolide (2 g.) was shaken with 10 c.c. of saturated hydrogen bromide solution for 18 hours and the product was poured into concentrated ammonium acetate solution. The precipitate was collected, washed thoroughly with water and then boiled with charcoal in methyl alcohol for $\frac{1}{2}$ hour. The filtered solution was cooled and poured into water. This process was repeated, when a hydrobromide was obtained as a nearly white powder, melting indefinitely between 117° and 124°. (Found: Br, 23.3. $C_{20}H_{31}O_8Br$ requires Br, 18.5 per cent).

(d) Andrographolide (2 g.) was dissolved in glacial acetic acid and the solution was saturated with hydrochloric acid gas. The solution was kept sealed in a tube overnight at the ordinary temperature and then poured into water containing ammonium acetate. The precipitate was collected, dissolved in rectified spirit, boiled with charcoal and filtered. The filtrate was cooled and poured into water. The precipitate was collected and dried *in vacuo* over calcium chloride and sodium hydroxide. A white crystalline powder, m. p. 56-57°, was obtained. (Found: Cl, 9.15. $C_{20}H_{31}O_8Cl$ requires Cl, 9.18 per cent).

(e) Andrographolide (0.4082 g.) was dissolved in glacial acetic acid by warming, cooled and to the solution was added Wijs solution [(25 c.c.) containing 0.8468 g. of iodine and 0.8069 g. of ICl_3]. The mixture after standing for 24 hours was titrated against 0.0996 *N*/1-thiosulphate after the addition of 20 c.c. of 10% potassium iodide and 100 c.c. of water. ICl equivalent to 23.55 c.c. of thiosulphate was used up. (Found: Iodine Value, 72.9. Calc. for one-double bond, 72.57).

Reduction of Andrographolide: Formation of two Isomeric Dihydro Derivatives.—To finely divided palladium prepared by shaking a concentrated aqueous solution of palladium chloride (1 g.), diluted with 25 c.c. of methyl alcohol, by hydrogen for $\frac{1}{2}$ hour, was added andrographolide (0.3026 g.) which rapidly dissolved in the medium and the mixture shaken for 2 hours in hydrogen atmosphere till the absorption of gas (21.1 c.c. at N.T.P.) was complete (one double bond requires 19.36 c.c.). The solution after removal of palladium was concentrated and then poured into much water, when

crystals of the dihydro derivative were obtained. After recrystallisation it had m.p. 205° (decomp.). (Found: C, 68.87; H, 9.69. $C_{20}H_{32}O_8$ requires C, 68.18; H, 9.18 per cent)

To a solution of andrographolide (2 g.) in concentrated hydrochloric acid (20 c.c.) an excess of crystallised stannous chloride was added and the mixture kept overnight and then poured into ice. The precipitated material crystallised from dilute alcohol in colourless needles, m.p. 200° (decomp.). (Found: C, 68.2; H, 10.27. $C_{20}H_{32}O_8$ requires C, 68.18; H, 9.18 per cent). A mixture of the two reduction products melted at $183-85^{\circ}$.

Tests for the Hydroxyl Group.—(a) Andrographolide was heated with an excess of acetic anhydride and fused sodium acetate for 1 hour and then poured into water. The white powder after purification gave C, 70.55%, H, 7.93%. A monoacetylandrographolide requires C, 67.2; H, 8.1 per cent. A dehydration product requires C, 72.3; H, 8.4 per cent. The analytical data agree with the formula $C_{40}H_{68}O_8$, which may be formed by loss of one molecule of water from two molecule of the substance. The nature of the product is not known

(b) Andrographolide (1 g.) on being shaken with phosphorus oxychloride (10 c.c.) gradually went into solution (more rapidly on warming). The dark greenish solution after standing for 12 hours was poured into excess of ice-water, when a nearly white flocculent mass separated. It was collected, washed repeatedly with water and dried in vacuum desiccator. It is easily soluble in benzene and alcohol and had m.p. $85-90^{\circ}$ (decomp.). The presence of phosphorus and chlorine was qualitatively demonstrated.

(c) Andrographolide (1 g.), dissolved in acetic anhydride (5 c.c.), was heated at 100° for $\frac{1}{2}$ hour with phenyl isocyanate (3 g.) with constant shaking. There was a vigorous reaction with evolution of gas but no solid separated out. Dilution of the cooled product with dry petroleum ether precipitated a white gummy solid. This was collected, washed with dry petroleum ether, dissolved in dry benzene and reprecipitated with petroleum ether. This process was repeated 5 times, when a white amorphous powder, melting indefinitely at $90-95^{\circ}$, was obtained after drying in vacuum desiccator. It is easily soluble in most organic solvents excepting petroleum ether and water.

Further work is in progress.

The authors are indebted to Prof. S. N. Bose, Dean of the Faculty of Science, Dacca University, for his encouragement, helpful criticism and suggestions.

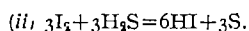
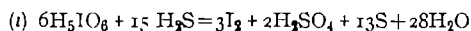
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Received March 23, 1939

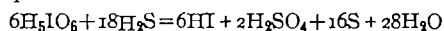
THE ACTION OF HYDROGEN SULPHIDE ON AN AQUEOUS SOLUTION OF PARAPERIODIC ACID.

BY R. K. BAHL AND SURJIT SINGH.

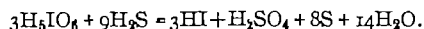
The action of hydrogen sulphide on paraperiodic acid may be represented by the following two partial equations —



The final equation is



or



The reaction starts according to the second equation after the first has been completed, *i. e.*, after the whole of paraperiodic acid has been reduced to iodine

In the course of a series of researches on the action of hydrogen sulphide on oxidising agents, such as chromates and permanganates, now in progress in this laboratory, attention was directed to its action on paraperiodic acid. Dunnicliff and his collaborators have also done work of a similar nature (Dunnicliff and Nijhawan, *J. Chem. Soc.*, 1926, 1; Dunnicliff and Soni, *J. Phys. Chem.*, 1929, 33, 81; Dunnicliff and Kotwani, *ibid.*, 1931, 35, 3214).

Hydrogen sulphide was prepared from ferrous sulphide and hydrochloric acid and was purified as described by Dunnicliff and Kotwani (*loc. cit.*). Paraperiodic acid was prepared by Partington and Bahl's method (*J. Chem. Soc.*, 1934, 1086).

Freshly prepared solution of paraperiodic acid (1%) of tested purity was used throughout. This reaction was not studied by bubbling the H_2S gas through the solution of paraperiodic acid as it resulted in the loss of free iodine liberated during the process.

A saturated solution of hydrogen sulphide in water was, therefore, added gradually to a solution of paraperiodic acid (1%). It resulted in the immediate liberation of free iodine and colloidal sulphur. The colour of the solution became deeper and deeper on account of the liberation of free iodine till a point was reached when the colour began to fade away and the solution turned milky.

The qualitative examination of the end-products obtained revealed the presence of (i) excess of free hydrogen sulphide, (ii) iodine ions due to the formation of hydroiodic acid, (iii) sulphate ions due to the presence of sulphuric acid and (iv) free sulphur which coagulated on standing.

The course of the reaction was investigated in stages. The products of the final stage differ from those of the initial stages in this respect that it contains hydroiodic acid, while the latter ones contain free iodine.

EXPERIMENTAL.

A saturated solution of hydrogen sulphide was prepared by passing the purified gas through distilled water, kept cool at a constant temperature by surrounding the bottle with melting ice. This ensured a constant concentration of the gas in the solution throughout the work.

The Reactions in the Intermediate Stages.

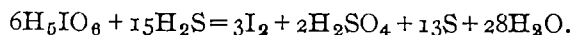
20, 40, 60 and 80 c.c. respectively of the above saturated solution of hydrogen sulphide were added to each 100 c.c. of 1% solution of paraperiodic acid. The temperature was again kept constant as in the preparation of H_2S solution. The products of the reaction were estimated as follows.

Iodine.—Iodine was estimated by extracting it with carbon tetrachloride and then titrating it against thiosulphate solution, in the presence of a strong solution of potassium iodide. Free sulphur extracted by carbon tetrachloride along with the iodine does not interfere.

Sulphur.—Sulphur was determined by coagulating it from a fresh solution of the same sample by the addition of ammonium chloride. It was then collected over a glass gooch sinter (Bagster, *J. Chem. Soc.*, 1928, 2631). Free iodine which settled along with the sulphur was washed out with potassium iodide solution.

Sulphuric acid was estimated as barium sulphate in the presence of an excess of hydrochloric acid, which dissolved any barium periodate formed by the action of barium chloride on unreacted paraperiodic acid.

Analytical results, given in Table I, are in agreement with the equation



The calculated values of sulphur for the stages A, B, and C are 9.99, 17.18 and 24.41% respectively, while those of sulphuric acid are 4.709, 8.095 and 11.502% respectively.

It is clear from Table I that the stage at which there is complete decomposition of the acid resulting in the complete liberation of iodine,

TABLE I.

Stage	I	II	III	IV	V	VI	VII
	Sat. soln. of H_2S added to 100 c. c. of 1% soln. of para-periodic acid	Sample No.	Iodine liberated	Acid decomposed (Calc. from free I_2)	Sulphur. pptd.	$BaSO_4$ formed	H_2SO_4 calc. from $BaSO_4$ in column VI
A	20 c. c.	1	18.288%	32.832%	9.72%	11.18%	4.783%
		2	18.350	32.943	9.66	11.46	4.812
		3	18.288	32.932	9.70	11.00	4.619
		Mean	18.308	32.869	9.69	11.28	4.704
B	40 c. c.	1	31.559	56.657	17.05	19.34	8.121
		2	31.496	56.544	17.56	19.28	8.096
		3	31.369	56.316	17.20	19.32	8.113
		Mean	31.474	56.505	17.27	19.31	8.110
C	60 c. c.	1	44.96	79.850	24.50	27.62	11.598
		2	44.45	79.800	24.56	27.58	11.581
		3	45.23	81.200	24.38	27.96	11.732
		Mean	44.88	80.283	24.48	27.72	11.637
D	80 c. c.	1	37.846	The whole of the acid has decomposed and I_2 has been further partially reduced to HI .	34.66	31.00	13.017
		2	37.465		35.10	30.82	12.945
		3	38.100		34.78	31.50	13.228
		Mean	37.803		34.84	31.10	13.063

lies somewhere between C and D and that at D, a part of the free iodine gets reduced to hydroiodic acid with the precipitation of free sulphur.

The Final Stage.

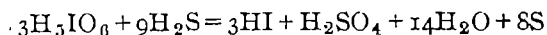
The final stage was reached by further addition of the saturated solution of hydrogen sulphide. It was found that 84 c. c. of the solution were required to effect the complete reduction of 100 c. c. of 1% paraperiodic acid solution. However, an excess of the H₂S solution was used to ensure the completion of the reaction. The system at the end consisted of (i) free hydrogen sulphide, (ii) hydroiodic acid, (iii) sulphuric acid, and (iv) free sulphur.

The solution on standing resulted in the coagulation of sulphur which was estimated as before. Free hydrogen sulphide was removed from the filtrate by the addition of an excess of cadmium carbonate. Sulphuric acid was estimated as barium sulphate and iodine was determined as silver iodide.

TABLE II.

Sample No.	Iodine.	Sulphur pptd.	BaSO ₄ , formed	H ₂ SO ₄ calc from BaSO ₄ .
1	55.290%	38.24%	31.02%	13.026%
2	55.540	38.10	31.40	13.185
3	55.469	37.46	31.48	13.218
4	55.361	37.84	31.42	13.194

These values agree with the equation



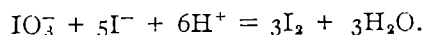
which requires I, 55.701; S, 37.42; and H₂SO₄, 14.32%.

POTENTIOMETRIC STUDIES IN OXIDATION REDUCTION REACTIONS. PART VI. IODOMETRIC DETERMINATION OF ORGANIC ACIDS.

BY BALWANT SINGH AND SOHAN SINGH.

Oxalic acid, tartaric acid, citric acid, malic acid and glycollic acid have been determined by the iodometric method. The liberated iodine was titrated potentiometrically against sodium thiosulphate solution at 10^4 , using a platinum electrode coupled with a saturated calomel electrode. E. M. F. was found to decrease steadily with the addition of standard sodium thiosulphate. At the equivalence point, there was a sharp fall in potential in each case.

When a solution of an acid is treated with iodide and iodate in excess, an equivalent quantity of iodine is liberated. The reaction may be represented by the equation



The iodine that is liberated can be titrated against standard sodium thiosulphate, thus affording a method for the determination of an acid. At higher hydrogen-ion concentrations the reaction proceeds practically instantaneously so that the method is well adapted to the determination of strong acids (Kolthoff, *Pharm. Weekbl.*, 1920, 67, 63).

In the case of moderately strong acids, like oxalic, tartaric and acetic, the theoretical amount of iodine is not liberated even after twenty-four hours' action with the iodate-iodide mixture (Groger, *Z. angew. Chem.*, 1890, 3, 353). Weaker acids cannot be titrated directly by this iodometric method because the hydrogen-ion concentration becomes too small at the end to permit the reaction to proceed quantitatively.

Bruhns (*Z. anal. Chem.* 1916, 55, 45) has shown that oxalic acid may be titrated after the addition of calcium or magnesium salt. The oxalate ions are precipitated and an equivalent amount of a strong mineral acid is liberated. Kolthoff ("Volumetric Analysis", 1929, Vol. II, p. 390) has found that the anions of the organic hydroxy-acids form complex ions with calcium, barium, magnesium or zinc salts. The acidity of the solutions is thereby increased. The organic hydroxy-acids are, therefore, capable of quantitative determination by the iodometric method.

In the present investigation oxalic acid, tartaric acid, citric acid, malic acid and glycollic acid have been determined potentiometrically by the iodometric method.

metric method using barium, zinc or magnesium salt as the precipitating agent.

EXPERIMENTAL.

A known weight of the organic acid was dissolved in water. The solution was mixed with barium, zinc or magnesium salt and an excess of potassium iodide and potassium iodate. The liberated iodine was titrated potentiometrically against standard sodium thiosulphate at 10° using a platinum electrode coupled with a saturated calomel electrode. The titrant was added from a burette and the mixture was kept stirred by a mechanical stirrer. A series of potentiometric titrations were performed with different amounts of each substance. One titration as typical of that set, is recorded in the following table.

TABLE I.

Titration of 0.0337 g. of tartaric acid dissolved in 20 c.c. of water, mixed with 10 c.c. of 10% BaCl_2 , 10 c.c. of $M/10\text{-KI}$ and 10 c.c. of $M/20\text{-KIO}_3$ against $M/50\text{-Na}_2\text{S}_2\text{O}_3$.

Thio.	E. M. F.	E/C (m volt/c.c.).	Thio	E. M. F.	E/C. (m volt/c.c.).
0.000 c.c.	0.400 volts		22.350 c.c.	0.296 volts	
		2			640
5.000	0.390		22.375	0.280	
		2			2800
10.000	0.382		22.400	0.210	(maximum)
		2			800
15.000	0.372		22.425	0.190	
		2			400
18.000	0.365		22.450	0.180	
		5			160
20.000	0.355		22.500	0.172	
		7			140
21.000	0.348		22.600	0.158	
		16			60
21.500	0.340		22.900	0.140	
		20			23
21.900	0.332		23.500	0.126	
		40			16
22.200	0.320		24.000	0.118	
		120			12
22.300	0.308		25.000	0.106	
		240			

DISCUSSION.

In these titrations, with the addition of standard sodium thiosulphate, the E. M. F. decreased steadily till the equivalence point. At the equivalence point there was a sharp fall in E. M. F. in each case. For the

addition of 0.05 c.c. of the titrant, the inflection potential was of the order of 80 to 108, 70 to 90, 70 to 84, 72 to 84 and 70 to 92 millivolts for oxalic acid, tartaric acid, citric acid, malic acid and glycollic acid respectively. after the equivalence point, there was again a fall in the E. M. F. which became steady on further addition of the reagent.

From the volume of sodium thiosulphate solution required in each titration, corresponding to the equivalence point, the amount of the substance was calculated. In the following table, the values obtained are compared with the amounts of the substance taken.

TABLE II.

Oxalic acid		Tartaric acid		Citric acid		Malic acid		Glycollic acid	
taken.	found	taken	found.	taken	found.	taken.	found.	taken.	found.
0.1890 g	0.1888 g	0.2251 g	0.2246 g.	0.1921 g	0.1917 g.	0.2011 g.	0.2013 g.	0.2282 g	0.2278 g
0.1323	0.1322	0.1575	0.1571	0.1344	0.1342	0.1407	0.1408	0.1026	0.1023
0.0567	0.0567	0.0675	0.0674	0.0864	0.0861	0.0904	0.0906	0.0684	0.0683
0.0378	0.0378	0.0450	0.0448	0.0576	0.0575	0.0603	0.0603	0.0456	0.0454
0.0252	0.0252	0.0337	0.0336	0.0384	0.0383	0.0301	0.0302	0.0342	0.0341

These results show that oxalic acid, tartaric acid, citric acid, malic acid and glycollic acid can be potentiometrically determined by the iodometric method.

The authors are indebted to Khalsa College authorities for a research grant and providing facilities for the work.

DEPARTMENT OF CHEMISTRY,
KHALSA COLLEGE, AMRITSAR.

Received June 13, 1939

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS. PART VII DETERMINATION OF AROMATIC COMPOUNDS WITH POTASSIUM CHLORATE.

BY BALWANT SINGH AND SOHAN SINGH.

Phenol, *p*-nitroaniline, diphenylamine and quinone have been determined at 25° by titrating them potentiometrically against standard potassium chlorate in presence of hydrochloric acid, using platinum electrode coupled with a saturated calomel electrode

The potentiometric method for the detection of the end-point in the titration of aromatic compounds with bromate in the presence of bromide, has been applied by Callan and Horrobin (*J. Soc. Chem. Ind.*, 1929, **47**, 334). No attempt has been made to study potentiometrically the chlorination of the organic compounds with suitable reagents.

In the present investigation, phenol, *p* nitroaniline, diphenylamine and quinone have been chlorinated by a solution of potassium chlorate in presence of hydrochloric acid.

EXPERIMENTAL.

A known weight of the organic compounds was dissolved in dilute hydrochloric acid. The solution was titrated potentiometrically against standard potassium chlorate at 25°, using a platinum electrode coupled with a saturated calomel electrode. The titrant was added very slowly from a burette and the mixture was kept thoroughly stirred by a mechanical stirrer.

Phenol, *p*-nitroaniline, diphenylamine and quinone were quantitatively converted into trichlorophenol (m.p. 68°), dichloronitroaniline (m. p. 189°), tetrachlorodiphenylamine (m. p. 133°) and tetrachloroquinone (m. p. 290° in a sealed tube) respectively.

A series of potentiometric titrations were performed with different amounts of each substance. One titration, as typical of that set, is recorded in the following table.

TABLE I

Titration of 0.0268 g. of quinone dissolved in 20 c. c. of water and 40 c.c. of conc. HCl against $M/60\text{-KClO}_3$.

KClO_3 .	E. M. F.	E/C (m. volts/c. c.)	KClO_3	E. M. F.	E/C (m. volts/c.c.),
0.000 c. c.	0.410 volts		19.850 c. c.	0.580 volts	
1.000	0.460	50	19.900	0.580	—
5.000	0.500	10	19.950	0.580	—
10.000	0.540	8	20.000	0.580	—
15.000	0.560	8	20.050	0.580	—
18.000	0.580	—	20.100	0.580	—
18.500	0.580	—	20.150	0.630	2200 (maximum)
18.800	0.580	—	20.200	0.750	1200
19.000	0.580	—	20.250	0.800	1000
19.200	0.580	—	20.350	0.840	400
19.400	0.580	—	20.650	0.872	107
19.600	0.580	—	21.150	0.894	44
19.800	0.580	—	22.150	0.920	26

DISCUSSION.

In these titrations, the E. M. F. rose on the first few additions of the titrant. On further addition of titrant, the potential remained constant till the equivalence point. At the equivalence point, there was a sharp jump in potential followed by a steady rise.

From the volume of potassium chlorate solution required in each titration, corresponding to the equivalence point, the amount of each compound was calculated. In the following table the values obtained are compared with the amounts of the substance taken.

TABLE II.

P h e n o l		<i>p</i> -Nitroaniline		Diphenylamine		Q u i n o n e	
taken.	found.	taken	found.	taken.	found.	taken	(found.
0'2121 g.	0'2118 g	0'3104 g	0'3098 g	0'1904 g	0'1901 g.	0'1213 g	0'1216 g
0'1275	0'1271	0'1728	0'1725	0'1271	0'1267	0'0806	0'0810
0'0568	0'0565	0'1554	0'1551	0'0955	0'0952	0'0604	0'0608
0'0426	0'0423	0'1035	0'1032	0'0644	0'0640	0'0402	0'0406
0'0356	0'0352	0'0595	0'0591	0'0425	0'0423	0'0268	0'0271

The results show that phenol, *p*-nitroaniline, diphenylamine and quinone can be potentiometrically determined by using standard potassium chlorate, in presence of hydrochloric acid.

The authors thank the Khalsa College authorities for a research grant and for providing facilities for the research work.

DEPARTMENT OF CHEMISTRY
KHALSA COLLEGE,
AMRITSAR.

Received July 7, 1939

STUDIES IN DEHYDROGENATION PART IV

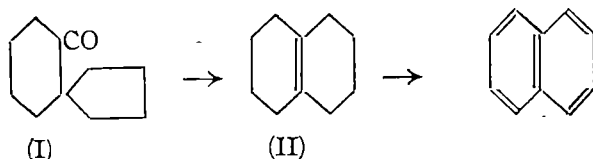
BY SURESH CHANDRA SEN-GUPTA.

In the present communication the syntheses of 7-methyl- and 7-ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spirocyclopentane have been described and the selenium dehydrogenation of these two spiro-hydrocarbons has been studied.

Interesting results were obtained when spiro-hydrocarbons were subjected to selenium dehydrogenation. It has been shown in a previous paper (*J. Indian Chem. Soc.*, 1934, **11**, 389) that tetrahydronaphthalene-spirocyclopentane (VII; R=H) undergoes ring transformation when heated with selenium, with the formation of both anthracene and phenanthrene. Now it has been found that 7-methyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VII; R=Me) gives on dehydrogenation with selenium both 3-methylphenanthrene and β -methylantracene, while 7-ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spirocyclopentane (VII; R=Et) yields 3-ethylphenanthrene and probably β -ethylantracene. The cyclopentane ring is not dehydrogenated till it opens up and is transformed into a six-membered ring. In all cases, studied so far, it has been observed that below 340° no appreciable ring transformation takes place and a temperature of 340-350° is most suitable for this change.

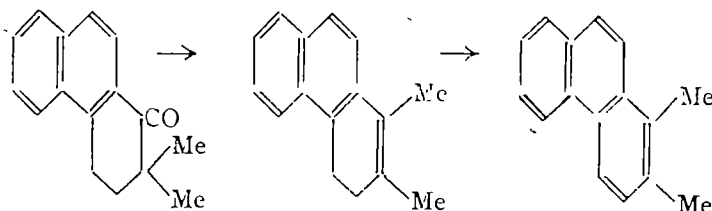
This ring transformation can be best explained by assuming that during dehydrogenation the fission of the cyclopentane ring takes place along the dotted lines (VII) giving rise to an intermediate which undergoes a dual mode of cyclisation with the production of both anthracene and phenanthrene derivatives.

An alternative view has been advanced by Linstead (*Ann. Rep. J. Chem. Soc.*, 1936, p. 304) based on retro-pinacolic change during the Clemmensen reduction of the spiro-ketone (I) or during its dehydrogenation with the production of (II). In spite of apparent simplicity of this explanation



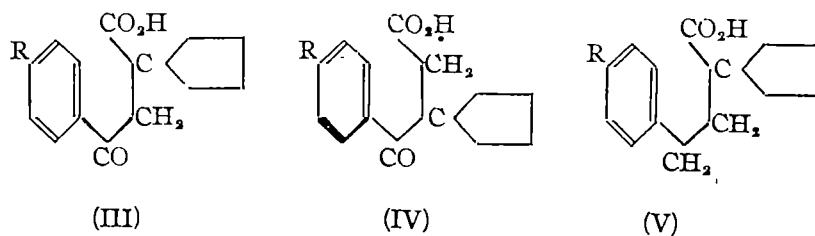
the issue is not quite clear, because it cannot adequately explain the simultaneous formation of phenanthrene and anthracene during the

dehydrogenation of tetrahydronaphthalene-spiro-cyclopentane (VII ; R=H). Furthermore, on the basis of Linstead's views gem-dialkylated tetralones and keto-phenanthrenes on reduction and dehydrogenation should give di-alkylnaphthalenes and phenanthrenes, which is contrary to experience.



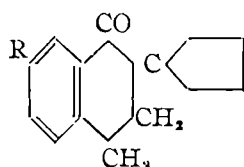
Actually the products isolated were mono-alkylnaphthalenes and phenanthrenes (*J. pr. Chem.*, 1938, **151**, 82 ; 1939, **152**, 9).

The two spiro-hydrocarbons (VII ; R=Me and R=Et) required for dehydrogenation studies were synthesised in the following manner. The anhydride of cyclopentane-1-carboxy-1-acetic acid condenses with toluene in presence of anhydrous aluminium chloride, with the formation of a keto-acid which must be either (III ; R=Me) or (IV ; R=Me). The keto-acid on reduction by the Clemmensen method gives a γ -tolylbutyric acid, the ethyl ester of which does not condense with ethyl oxalate in presence of potassium ethoxide, indicating absence of hydrogen in the α -carbon atom. The constitution of the tolylbutyric acid should, therefore, be represented by (V ; R=Me) and consequently the keto-acid by (III ; R=Me). It is found that the chloride of the half ester (VIII) (Bardhan, *J. Chem. Soc.*, 1928, 2593) reacts with toluene in presence of aluminium chloride giving a keto-ester which on hydrolysis forms a keto-acid identical with (III ; R=Me), instead of the expected acid (IV ; R=Me).

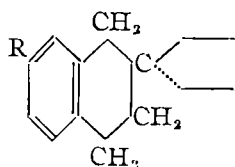


In order to explain this observation a rearrangement of the acid chloride during the Friedel-Craft's reaction has to be assumed.

The keto-acid obtained by the condensation of benzene and the anhydride of *cyclopentane-1-carboxy-1-acetic acid* (*J. Indian Chem. Soc.* 1934, 11, 392), to which the formula (IV; R=H) was attributed, has now been proved to be *αα-cyclopentane-β-benzoylpropionic acid* (III; R=H).



(VI)



(VII)



(VIII)

The cyclisation of *αα-cyclopentane-γ-(p-tolyl)-butyric acid* (V; R=Me) with 85% sulphuric acid gives the spiro-ketone (VI; R=Me), (*semicarbazone*, m.p. 141-42°) which on reduction according to Clemmensen gives 7-methyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VII; R=Me). The latter on dehydrogenation with selenium gives both 3-methylphenanthrene and *β*-methylanthrane. The formation of 3-methylphenanthrene from the spiro-hydrocarbon (VII; R=Me) lends additional support to the constitution of the keto-acid (III, R=Me). The keto-acid with the alternative structure (IV; R=Me) would have furnished 2-methylphenanthrene and *β*-methylanthrane as the final products of dehydrogenation.

In a similar manner 7-ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VII, R=Et) has been synthesised, starting from the anhydride of *cyclopentane-1-carboxy-1-acetic acid* and ethyl benzene. These two condense in nitrobenzene solution in presence of aluminium chloride giving *αα-cyclopentane-β-(p-ethyl)-benzoylpropionic acid* (III; R=Et). The constitution of this keto-acid also follows from the observation that the ethyl ester of *αα-cyclopentane-γ-(p-ethyl) phenylbutyric acid* (V, R=Et), obtained by the Clemmensen reduction of the keto acid (III; R=Et) did not form any oxalyl compound. The *γ*-arylbutyric acid is cyclised with 85% sulphuric acid giving a 76% yield of 1-keto-7-ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spirocyclopentane (VI, R=Et). The spiro-ketone on reduction by the Clemmensen method gives the desired spiro-hydrocarbon (VII; R=Et). This on dehydrogenation with selenium gives 3-ethylphenanthrene and probably *β*-ethylanthracene. The isolation of these dehydrogenation products further proves the position of the *cyclopentane* ring in the *αα*-position of the keto-acid (III; R=Et).

E X P E R I M E N T A L.

αα-cyclopentane-β-(p-toluoyl)-propionic Acid (III; R=Me).—(a) Aluminium chloride (52 g.) was added to a solution of the anhydride of cyclopentane-1-carboxy-1-acetic acid (30 g.) in dry toluene (100 c.c.) cooled in ice. The mixture was kept at room temperature for 12 hours and warmed at 60-65° for 3 hours. The product was treated with ice and excess of toluene removed in steam. The solid product was purified by extraction with sodium carbonate solution and was crystallised from dilute alcohol in plates, m. p. 149-50°, yield 38 g. (Found: C, 73.4; H, 7.6. $C_{15}H_{18}O_3$ requires C, 73.2; H, 7.3 per cent).

The semicarbazone of this keto-acid was prepared in aqueous methyl alcoholic solution. It crystallised from alcohol in needles, m. p. 164-65°. (Found: C, 63.5; H, 6.7. $C_{16}H_{21}O_3N_3$ requires C, 63.4; H, 6.9 per cent).

(b) Aluminium chloride (18 g.) was added to a solution of the acid chloride of methyl cyclopentane-1-carboxy-1-acetate (11 g.) in excess of toluene (40 c.c.) cooled in ice. After keeping at the room temperature for 12 hours the mixture was treated with ice, excess of toluene distilled off in steam and the oily product extracted with ether. The ether extract was washed with sodium carbonate solution, dried and distilled as a thick oil, b. p. 170-75°/5 mm. (Found: C, 73.6; H, 7.9. $C_{16}H_{20}O_3$ requires C, 73.8; H, 7.7 per cent).

It was hydrolysed with alcoholic potash and the resulting keto-acid crystallised from dilute alcohol, m. p. 149-50°.

αα-cyclopentane-γ-(p-tolyl)-butyric Acid (V; R=Me).—*αα*-cyclopentane-β-(p-toluoyl)-propionic acid (15 g.), amalgamated zinc (75 g.) and concentrated hydrochloric acid (75 c.c.) were boiled for 24 hours. The reduced acid was extracted with ether, ether distilled off and the residue was dissolved in warm sodium carbonate solution, filtered and acidified with hydrochloric acid. The separated reduced acid (sticky solid) was purified by distillation under reduced pressure, b. p. 186-90°/5 mm. The liquid distillate readily solidified and was crystallised from petroleum ether (b. p. 40-60°) in colourless needles, m. p. 68-69°, yield 10.5 g. (Found: C, 77.6; H, 8.6. $C_{16}H_{20}O_2$ requires C, 77.6, H, 8.6 per cent).

The *anilide* crystallised from dilute alcohol in needles, m. p. 124° . (Found. C, 82.0 ; H, 8.1 . $C_{21}H_{15}ON$ requires, C, 82.1 ; H, 8.1 per cent).

The *ethyl ester* was prepared by the action of ethyl alcoholic hydrogen chloride. It is a colourless oil, b. p. $160-62^{\circ}/5$ mm. (Found: C, 78.2 ; H, 9.1 . $C_{17}H_{24}O_2$ requires C, 78.5 , H, 9.2 per cent).

7-Methyl-1-keto-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VI; R = Me).— α -cyclopentane- γ -(*p*-tolyl)-butyric acid (15 g.) was added to 85% sulphuric acid and the mixture heated at 100° for $1\frac{1}{2}$ hours with stirring. The product was poured on ice, extracted with ether, the extract washed with dilute ammonia and water, dried with sodium sulphate and distilled, b. p. $160-63^{\circ}/5$ mm. It possesses a characteristic smell, yield 11 g., i.e., 77% of the theoretical, d_4^{20} , 1.0578 ; n_D , 1.55583 ; $[R_L]_D$, 65.0 (calc., 63.5). (Found. C, 83.8 ; H, 8.5 . $C_{15}H_{18}O$ requires C, 84.1 ; H, 8.4 per cent).

The *semicarbazone* was obtained by heating the spiro-ketone with semicarbazide acetate in methyl alcoholic solution for 6 hours. After distilling off the alcohol, the residue was triturated with a little petroleum ether when the semicarbazone was obtained in a crystalline state. It crystallised from dilute alcohol in fine needles, m. p. $141-42^{\circ}$. (Found: C, 70.8 ; H, 7.6 . $C_{16}H_{21}N_3$ requires C, 70.8 ; H, 7.7 per cent).

7-Methyl-1:2:3:4-tetrahydronaphthalene-2:3-spiro-cyclopentane (VII; R = Me).—The foregoing spiro-ketone (8 g.) was boiled with amalgamated zinc (40 g.) and concentrated hydrochloric acid (40 c.c.) for 24 hours. The product was extracted with ether, washed, dried and distilled, when a colourless liquid (5 g.) came over at $135-36^{\circ}/6$ mm, d_4^{21} , 0.980417 ; n_D , 1.53856 ; $[R_L]_D$, 63.86 (calc., 63.47). (Found: C, 89.7 ; H, 10.2 . $C_{15}H_{20}$ requires C, 90.0 ; H, 10.0 per cent).

Selenium Dehydrogenation of 7-Methyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane.—The spiro-hydrocarbon (3 g.) was heated with powdered selenium (6 g.) in a metal-bath at $300-340^{\circ}$ for 6 hours and at $340-50^{\circ}$ for 24 hours. The product was extracted with ether, the solvent removed and the residual oil distilled over sodium. At first a liquid fraction (1.5 g.) came over at $140-50^{\circ}/6$ mm. and then a small amount at a higher temperature, solidifying in the side-tube of the distilling flask and exhibit-

ing a bluish fluorescence. The solid fraction was pressed on a porous tile and had m. p. $198-200^{\circ}$ and this was probably β -methylanthracene. The liquid fraction was warmed with picric acid in alcoholic solution and the separated picrate after recrystallisation from spirit had m. p. 135° . The hydrocarbon was regenerated from the picrate and after crystallisation from alcohol melted at $61-62^{\circ}$. (Found: C, 93.5; H, 6.4. Calc. for $C_{15}H_{12}$: C, 93.75; H, 6.25 per cent).

The *picrate* prepared from this regenerated hydrocarbon had m. p. $137-38^{\circ}$ and the mixed m. p. with the picrate of an authentic sample of 3-methylphenanthrene showed no depression.

α-cyclopentane-β-(p-ethyl)-benzoylpropionic Acid (III; R = Et).—

(a) It was prepared from ethyl benzene (25 g.), anhydride of cyclopentane-1-carboxy-1-acetic acid (31 g.), carbon disulphide (100 c.c.) and aluminium chloride (52 g.) according to the procedure followed in the case of *α-cyclopentane-β-(p-toluoxy)-propionic acid*. It was crystallised from petroleum ether (b. p. $90-100^{\circ}$) in needles, m. p. $128-29^{\circ}$, yield 38 g. (Found: C, 73.7; H, 7.7. $C_{16}H_{20}O_3$ requires C, 73.8; H, 7.7 per cent).

The *semicarbazone* crystallised from dilute alcohol in needles, m. p. 130° . (Found: C, 64.1; H, 7.3. $C_{17}H_{23}O_3N_3$ requires C, 64.3; H, 7.3 per cent).

(b) The chloride prepared from 5 g. of methylcyclopentane-1-carboxy-1-acetate and 4 g. of ethylbenzene in 30 c.c. of carbon disulphide were treated with anhydrous aluminium chloride (7.5 g.). The product, a keto-ester, was worked in the usual manner and distilled as a thick oil at $195-98^{\circ}/10$ mm, yield 4.9 g. (Found: C, 74.2; H, 8.1. $C_{17}H_{22}O_3$ requires C, 74.4; H, 8.0 per cent).

This keto-ester on hydrolysis with alcoholic potash gave an acid which after crystallisation from petroleum ether melted at $128-29^{\circ}$ and the mixed m. p. with a sample prepared by method (a) remained undepressed.

α-cyclopentane-γ-(p-ethyl)-phenylbutyric Acid (V; R = Et).—The foregoing keto-acid (30 g.) was reduced by the Celemmensen method with amalgamated zinc (150 g.) and concentrated hydrochloric acid (150 c.c.). The reduced acid (solid) was first purified by extraction with sodium carbonate solution and finally by distillation at $200-02^{\circ}/9$ mm. This distillate slowly solidified and crystallised from petroleum ether (b. p. $30-50^{\circ}$) in thick

needles, m. p. 51-53°, yield 21 g. (Found: C, 77·8; H, 9·0. $C_{16}H_{22}O_2$ requires C, 78·2; H, 8·9 per cent).

The *anilide* crystallised from dilute alcohol in needles, m. p. 117°. (Found: C, 82·0; H, 8·4. $C_{22}H_{27}ON$ requires C, 82·2; H, 8·4 per cent).

The *ethyl ester* was prepared by the action of ethyl alcoholic hydrogen chloride. It is a colourless oil, b. p. 144-46°/5 mm. (Found: C, 78·6; H, 9·6. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·5 per cent). Ethyl oxalate did not condense with the ethyl ester in presence of potassium ethoxide.

1-Keto-7-ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VI; R=Et).—*αα-cyclopentane-γ-(p-ethyl) phenylbutyric acid* (15 g.) was cyclised with concentrated sulphuric acid (45 c.c.) and water (15 c.c.) at 100°. The product was a colourless liquid, b.p. 175°/9 mm., yield 10·5 g., $d_4^{28.5}$, 1·04336; n_D , 1·552907; $[R_L]_D$, 69·9. (Found: C, 84·9; H, 8·9. $C_{18}H_{20}O$ requires C, 84·2; H, 8·8 per cent).

7-Ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane (VII; R=Et).—The foregoing spiro-ketone (9 g.) was reduced by heating with amalgamated zinc (50 g.) and concentrated hydrochloric acid (50 c.c.) for 24 hours. The product distilled at 154-56°/9 mm., yield 7·2 g., $d_4^{27.8}$, 0·96652; n_D , 1·53515; $[R_L]_D$, 68·95. (Found: C, 89·5; H, 10·2. $C_{18}H_{22}$ requires C, 89·7; H, 10·3 per cent).

Selenium Dehydrogenation of 7-Ethyl-1:2:3:4-tetrahydronaphthalene-2:2-spiro-cyclopentane.—The spiro-hydrocarbon (4·5 g.) was heated with selenium (5 g.) in a metal-bath at 300-20° for 6 hours and at 340-50° for 24 hours. The product was extracted with ether and distilled over sodium under reduced pressure when 1·2 g. of an oily product containing a little crystalline substance was obtained. This was redistilled and the fractions coming at 170-75°/5 mm. and at 175-190°/5 mm. were separately collected.

The first fraction (0·8 g.) was warmed with picric acid (1 g.) in alcoholic solution and the picrate after being freed from oily matter by pressing on a porous tile, was twice recrystallised from spirit in orange needles, m. p. 117-18°. The mixed m. p. with the picrate of 3-ethyl phenanthrene, prepared from 3-phenanthroylmethyl ketone (Haworth and Mavin, *J. Chem. Soc.*, 1933, 1012), was 117-18°. (Found: C, 60·6; H, 4·0. Calc. for $C_{22}H_{17}O_7N_3$: C, 60·7; H, 3·9 per cent). The hydrocarbon regenerated from the picrate (0·5 g.) was distilled over sodium, the distillate (0·18 g.) did not solidify even on cooling. (Found: C, 93·1; H, 6·7. Calc. for $C_{16}H_{14}$: C, 93·2; H, 6·8 per cent).

The second fraction was cooled in ice when colourless shining flakes, m. p. $135-37^{\circ}$, separated. From its high melting nature it seems to be 2-ethylanthracene

The author desires to express his grateful thanks to Dr. M. Q. Khuda for giving facilities to carry out this work in the Presidency College Chemical laboratory, Calcutta and to Dr. J. C. Bardhan for his kind interest in this investigation.

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Received June 24, 1939

SPACE-GROUP DETERMINATION OF THE CRYSTALS OF p-NITROPHENOL (METASTABLE), PHENACETIN AND TRIBENZYLAMINE.

BY MATA PRASAD, JAGDISH SHANKER AND PRABHAKAR N. BALJEKAR.

The paper describes the results of space-group determination of the crystals of *p*-nitrophenol (metastable), phenacetin and tribenzylamine. Dimensions of the unit cells of the three crystals have been accurately determined by taking X-ray rotation photographs. All the three crystals belong to the space-group C_2^2 with Γm Bravis lattice. The molecules in the unit cell are asymmetric in each case.

In the study of these crystals K-radiation from a copper anticathode was used. The lengths of the axes and the abnormal halvings were respectively determined by taking rotation photographs and oscillation photographs at an interval of 10 or 15 degrees about the three principal axes of the crystals.

p-Nitrophenol (metastable).

The crystals develop the following faces

$$m(110), m'(\overline{110}), q(011) \text{ and } q'(0\overline{11}).$$

They belong to the monoclinic prismatic class and the axial ratio found by crystallographic measurements is

$a : b : c = 1.3836 : 1 : 0.3398$ and $\beta = 106^\circ 55'$ (*cf.* Groth, "Chemische Krystallographie" Vol. IV, p. 106).

The dimensions of the unit cell calculated from the rotation photographs (*cf.* Plate I) are :

$$a = 15.34 \text{ \AA}, \quad b = 11.15 \text{ \AA}, \quad c = 3.79 \text{ \AA}.$$

These give the axial ratio to be $a : b : c = 1.376 : 1 : 0.3399$ which is in good agreement with that obtained by crystallographic measurements.

Tables I and II give the list of reflecting planes with their relative intensities estimated by eye.

TABLE I.

Axial planes.	P r i s m p l a n e s				
	(hol).	(okl).	(hko).	(hko).	(hko).
001 m.s.	201 w.	011 v.s.	110 m.s.	250 v.w.	520 m.s.
002 m.s.	201̄ m.s.	012 w.m.	120 v.s.	260 w.	530 m.
020 m.s.	202̄ v.w.	021 m.s.	130 m.s.	320 v.w.	540 w.
040 m.s.	401 w.m.	051 m.s.	140 m.s.	340 w.	610 w.m.
200 m.	401̄ w.m.		150 w.	410 m.	630 w.m.
400 s.	601 v.w.		160 w.	420 s.	640 w.m.
600 w.m.	801̄ w.m.		210 s.	430 m.	710 w.m.
800 w.			220 v.s.	440 w.	720 w.
			230 m.	450 w.	730 w.
			240 w.m.	510 m.s.	810 w.
					820 w.

TABLE II.

General planes.

Plane.	Intensity.	Plane.	Intensity.	Plane.	Intensity.	Plane.	Intensity
111	v.s.	221̄	w.	411	w.m.	531	w.
111̄	v.s.	231	m.s.	411̄	m.s.	541̄	w.
121	w.m.	231̄	m.	412̄	v.w.	621	v.w.
121̄	w.	241	w.	421	w.	631̄	v.w.
131̄	m.s.	251	w.	421̄	w.	711̄	w.
141	m.	251̄	w.	422̄	w.	721̄	w.m.
141̄	w.	311̄	w.m.	431	w.	731̄	w.m.
211	m.	321̄	w.	431̄	m.	811̄	w.
211̄	m.	322̄	m.	511̄	m.	821̄	w.
212̄	v.w.	331	m.	512̄	v.w.		
221	m.	331̄	m.s.	521̄	m.s.		

It will be seen from the above that planes (hol) are halved when h is odd and (oro) is also halved. These halvings assign the crystal to the space-group C_{2h}^2 , which requires four asymmetric molecules. The number of molecules calculated from the dimensions of the unit cell and the specific gravity of the crystals, which was found to be 1.495, is also four (accurately 4.018). This shows that the molecules of the metastable form of *p*-nitrophenol in the unit cell are asymmetric.

Phenacetin.

The crystals develop the following faces :—

$a(100)$, $c(001)$, $m(110)$, $r(101)$, $s(102)$, $q(011)$, $t(021)$, etc.

They belong to the monoclinic prismatic class and the axial ratio determined by crystallographic measurements is

$a : b : c = 1.4213 : 1 : 0.8054$ and $\beta = 109^\circ 17'$ (*cf.* Groth, *ibid.*, p. 242).

The dimensions of the unit cell calculated from the rotation photographs (*cf.* Plate II) are :—

$$a = 13.78 \text{ \AA}, \quad b = 9.73 \text{ \AA}, \quad c = 7.82 \text{ \AA}.$$

These give the axial ratio $a : b : c = 1.416 : 1 : 0.804$ which is in good agreement with that mentioned above.

Tables III and IV give the reflecting planes observed in the crystal along with their approximate relative intensities.

TABLE III.

Axial planes.	P r i s m p l a n e s			
	(hol)	(okl).	(hko).	(hko).
100 s.	102 m.s.	012 v.s.	110 v.s	330 m s.
200 v.s.	202 v.s.	021 v.s.	120 w.	340 m.
300 m.	202 m.s.	022 m.s.	140 w.	410 m.s.
400 m s.	204 w.	023 m.	210 w.m.	420 s.
500 v.s.	302 m s.	031 s.	220 s.	440 m
600 s.	502 m.s.	032 w.	230 v.w.	510 m
040 m.	602 s.	033 m.	240 w.m	520 w.
002 m s.		041 m.	310 s.	610 v.
		42 m.	320 v.w.	620 m.
			710 m.s	
			720 m.	
			730 w m.	

TABLE IV.

General planes.

Plane.	Intensity.	Plane.	Intensity	Plane.	Intensity.	Plane.	Intensity
111	v s.	221	m.	321	m.s.	431	m.s.
11 $\bar{1}$	v s.	22 $\bar{1}$	m.	32 $\bar{1}$	v.w	43 $\bar{1}$	w
11 $\bar{2}$	w.	22 $\bar{2}$	m.	32 $\bar{2}$	m.s.	43 $\bar{2}$	m.s.
11 $\bar{3}$	w m.	22 $\bar{3}$	w.	32 $\bar{3}$	m.	43 $\bar{3}$	m.
12 $\bar{1}$	m.	22 $\bar{3}$	v.w	32 $\bar{4}$	v.w	44 $\bar{1}$	v.w
12 $\bar{2}$	m.s.	231	w.m	33 $\bar{1}$	m.s.	44 $\bar{1}$	w
12 $\bar{2}$	m s.	23 $\bar{1}$	m.s.	33 $\bar{2}$	w.	44 $\bar{2}$	w.
131	v s.	23 $\bar{2}$	w	33 $\bar{2}$	m s.	511	v.w
13 $\bar{1}$	m s.	23 $\bar{2}$	m.	34 $\bar{1}$	w m.	51 $\bar{1}$	w.m.
13 $\bar{2}$	m	23 $\bar{3}$	m	34 $\bar{1}$	m.	51 $\bar{2}$	m.s.
13 $\bar{2}$	w	241	w.	35 $\bar{1}$	m.	51 $\bar{3}$	m.s.
141	m.	24 $\bar{1}$	m	41 $\bar{1}$	v.s	52 $\bar{2}$	m.
14 $\bar{1}$	w m.	311	w.	41 $\bar{2}$	w.	52 $\bar{3}$	v.w.
14 $\bar{2}$	v.w	31 $\bar{1}$	m.s.	41 $\bar{2}$	m.	61 $\bar{1}$	v.w
211	m s.	31 $\bar{2}$	v.w.	41 $\bar{3}$	m s	61 $\bar{2}$	w.m.
21 $\bar{1}$	v.s.	31 $\bar{3}$	w.m	41 $\bar{3}$	m.	62 $\bar{1}$	m.
21 $\bar{2}$	w.m.	31 $\bar{3}$	m s	421	m.	62 $\bar{2}$	w.
21 $\bar{2}$	v w.	31 $\bar{4}$	m.s.	42 $\bar{1}$	m.	63 $\bar{2}$	w.
213	w			42 $\bar{2}$	m	71 $\bar{1}$	m.s.
				423	m.	71 $\bar{2}$	w.m.
				42 $\bar{3}$	m	71 $\bar{3}$	w.m

It will be seen that planes (hol) are halved when l is odd and (oro) is also halved. The crystal therefore belongs to the space-group C_{2h}^3 which requires four asymmetric molecules. The number of molecules calculated from the dimensions of the unit cell and the specific gravity of the crystal, found to be 1.202, is also four (accurately 4.00). This shows that the molecules of phenacetin in the unit cell are asymmetric.

Tribenzylamine.

The crystals of tribenzylamine have been found to develop the following faces:—

$$a(100), c(001), r(101), r'(10\bar{1}).$$

They belong to the monoclinic prismatic class and the axial ratio found by crystallographic measurements is $a : b : c = 1.2242 : 1 : 1.0130$; and $\beta = 95^\circ 4'$, (*cf.* Groth, *ibid.*, Vol. V, p. 323).

The dimensions of the unit cell calculated from the rotation photographs (Plate III) are

$$a = 22.03 \text{ \AA}, \quad b = 8.92 \text{ \AA}, \quad c = 9.04 \text{ \AA}.$$

These gives the axial ratio $a : b : c = 2(1.234) : 1 : 1.014$.

This shows that the a -axis is doubled, that is, has twice the length expected from the ratio obtained by the crystallographers.

Tables V and VI give the list of the reflecting planes in the crystal, along with their approximate relative intensities.

TABLE V.

Axial planes	Prism planes				
	(hol).	thol.	(okl)	(hko).	(hko).
001 s.	201 m.s.	601 m s	011 w.m.	110 m.s.	520 m.
002 s.	20 $\bar{1}$ w.	601 m.	012 s.	120 v.s.	530 v w.
003 w	202 s	602 w	013 v.w.	130 m	540 v.w
004 w.	203 m	60 $\bar{2}$ w	021 s.	210 s	610 m.s.
020 m s.	203 m.	603 m s.	022 w.	220 m.s.	620 m
200 v s.	401 s	60 $\bar{3}$ w.m.	023 v w	230 m.	630 v.w.
400 m.	401 s	801 m	024 w.	240 v.w.	710 m.s.
600 m	403 w	801 w	031 w.	410 s	720 w
800 w.m.	404 w.	(10)01 w m	033 w	510 m.s.	730 v.w
(10)00 v w.		(10)02 w.			810 m
					830 v.w.
					(10)10 w.
					(11)10 v.w.

TABLE VI.

General planes.

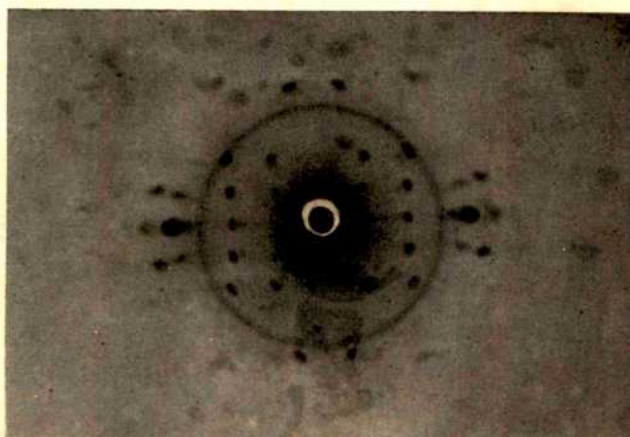
Plane	Intensity.	Plane	Intensity.	Plane	Intensity
111	m s.	223	m.	422	v w.
111	m s.	231	m s.	422	w.
112	s.	231	w.	423	m s.
112	m.s	232	v.w.	423	w.m
114	w.	232	w m.	431	m
114	w.m	233	w.	432	m.
121	w.	311	v.s.	433	w.
121	w m	311	v.s	511	m.s.
122	w.	312	m.s.	512	w m.
122	m	312	m s.	513	m.
123	w.	313	m.	521	w m
123	w.	321	m.s	521	w
124	w	321	s	522	m.
131	m.s	322	m s.	522	m
131	w	322	w m	523	w.
132	v.w.	323	w.	523	w m.
133	w.	324	v w	524	v.w.
211	v s.	332	v w.	524	v w.
211	v s.	333	w	531	w.
212	m.	333	m.	532	w.
212	m	341	v w	532	w.
213	w.	411	v.s	533	w.
213	m.	411	v s	542	w.m.
221	m s.	412	w m	611	w
221	m.s.	412	m s.	611	m.
222	w.m	413	w	612	w.m.
222	m.	421	m.s.	612	w.
223	w.m.	421	s	613	w.

PRASAD, SHANKER AND BALJEKAR

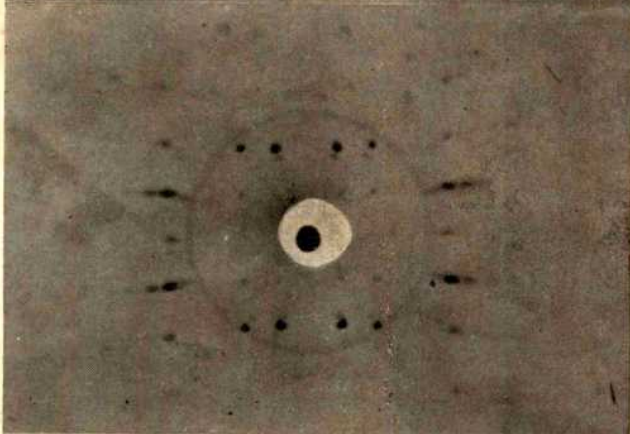
PLATE I.

• *Rotation photograph of p-Nitrophenol (metastable).*

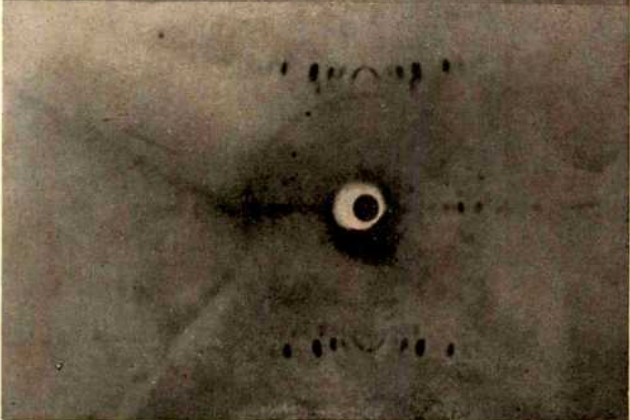
a-axis



b-axis



c-axis

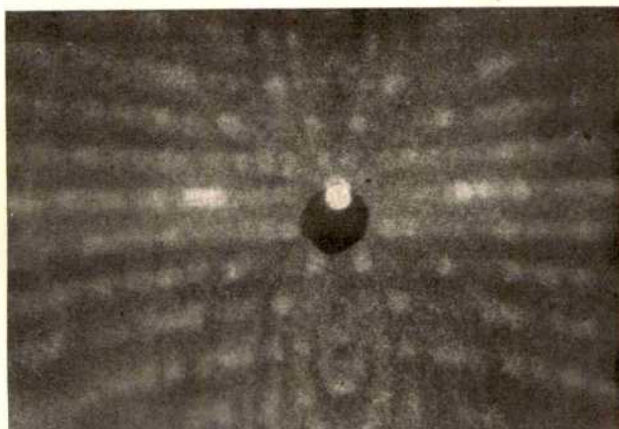


PRASAD, SHANKER AND BALJEKAR

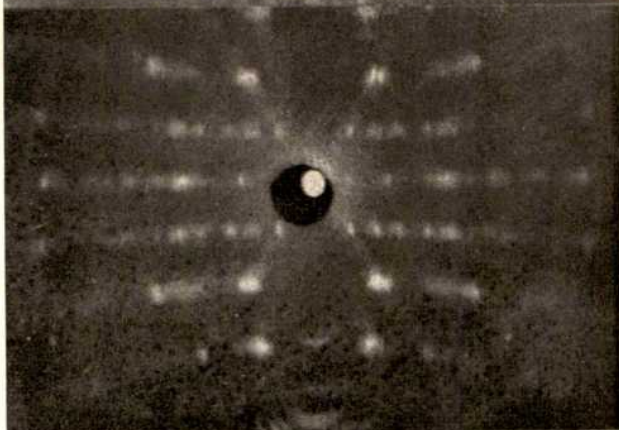
PLATE II.

Rotation photograph of phenacetin.

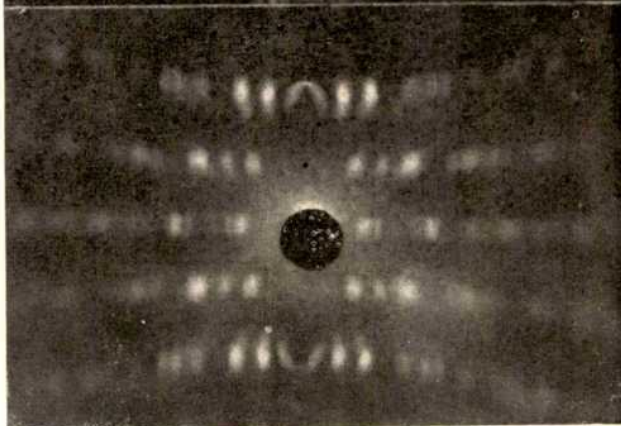
a -axis



b -axis



c -axis

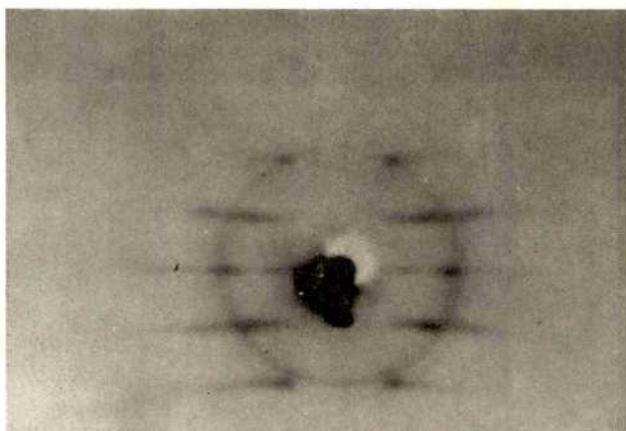


PRASAD, SHANKER AND BALJEKAR

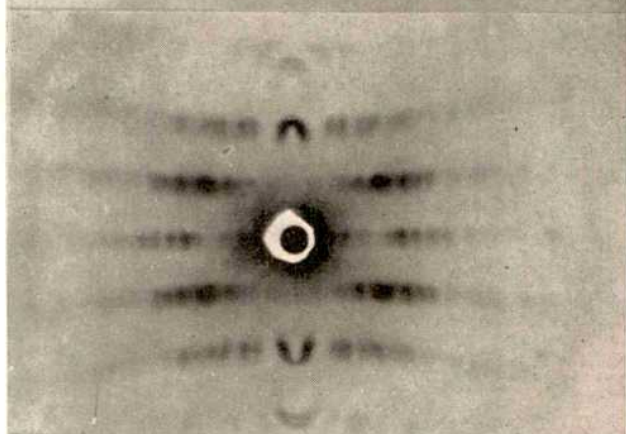
PLATE III.

Rotation photograph of tribenzylamine.

a-axis



b-axis



c-axis

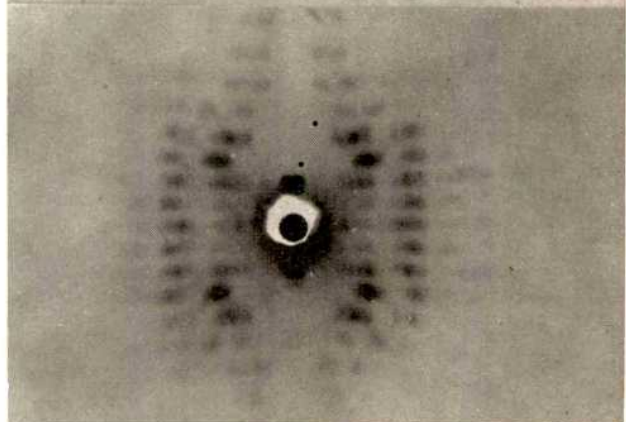


TABLE VI (*contd.*).

Plane.	Intensity	Plane	Intensity	Plane,	Intensity.
$61\bar{3}$	w.m	$71\bar{4}$	w.	822	w
621	v w	721	v w	831	w.
622	v.w.	$72\bar{1}$	v w	$83\bar{1}$	w.m
$62\bar{2}$	w	722	v w.	$83\bar{2}$	w m
$62\bar{3}$	w.	$72\bar{2}$	w m	911	w
$62\bar{4}$	w.	723	v.w	$91\bar{1}$	w
632	w	731	v w	$91\bar{2}$	w
641	v w	$73\bar{2}$	w.m	913	m.
$64\bar{1}$	v w.	811	m	$91\bar{3}$	w.
711	w	$81\bar{1}$	m s.	921	v w.
$71\bar{1}$	w.	812	v w.	$92\bar{1}$	v.w.
712	w	$81\bar{2}$	v w	$92\bar{2}$	v w.
$71\bar{2}$	w m	813	v.w.	$93\bar{1}$	w
713	m.	$82\bar{1}$	v w.	$(11)\bar{1}2$	v.w.

It will be seen that the planes (hol) are halved when h is odd and (oro) is also halved. The crystal, therefore, belongs to the space-group C_{2h}^1 which requires four asymmetric molecules. The number of molecules calculated from the dimensions of the unit cell and the specific gravity of the crystals which was found to be 1.074, is also four (accurately 3.99). This shows that the molecules of tribenzylamine in the unit cell are asymmetric.

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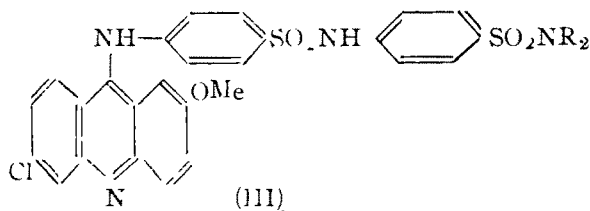
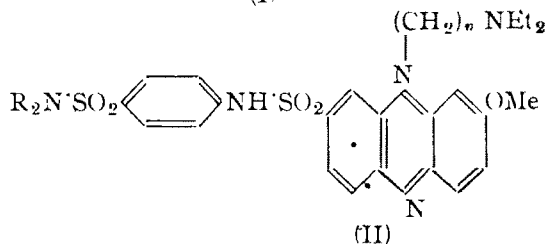
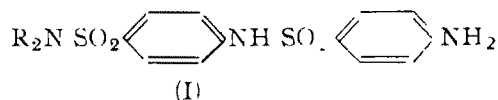
Received July 3, 1939

ACRIDINE DERIVATIVES AS ANTIMALARIALS PART IV.

By S. J. DAS-GUPTA.

Certain sulphonamidophenylsulphonamido-5-dialkylaminoalkylaminoacridines and 5-acridylsulphanilysulphanilamides have been prepared and described. Unlike other acridine derivatives they are comparatively tasteless, but possess, particularly in neutral solutions, the characteristic fluorescence of the 5-aminoacridines.

The sulphonamido-dialkylaminoalkylaminoacridine derivatives, particularly, 3-phenylsulphonylamido-7-methoxy-5-(δ -diethylaminobutyl and ω -diethylamino-isoamyl)-aminoacridines, recently described by Basu and Das-Gupta in Part III* of this investigation (*J. Indian Chem. Soc.*, 1939, **16**, 100) have since been found to possess bacteriostatic action against certain haemolytic streptococci, and also to be toxic to paramecium. It is known (*cf.* Rosenthal *et al.*, *Pub. Health Reports*, U. S. Treas. Dept., 1937, **52**, 662, Fourneau *et al.*, *Compt. rend. Soc. Biol.*, 1936, **122**, 258; Whitby, *Lancet*, 1938, *ii*, 1095) that the range of activity of any sulphanilamide derivative may be considerably widened by substituting the amino hydrogen by other grouping, such as sulphonamidophenyl [*cf.* the drug, "Uleron" (I, R=Me)]. Accordingly, certain acridine derivatives of the types (II) and (III) have been synthesised and described in the experimental part of this paper. Their bactericidal and antiparasitic actions are being studied.



* This part has been already printed as Part II.

E X P E R I M E N T A L.

2-Chloro-5-sulphonyl-(p'-sulphonamidophenyl)-aminobenzoic Acid.—1 Mol. of 1-carboxy-2-chlorobenzene-5-sulphonyl chloride (Basu and Das-Gupta, *loc. cit.*) and 2 mols. of *p*-aminobenzenesulphonamide were rubbed together with a little water in a mortar for about 2 hours and sodium carbonate was added to the mixture to keep it alkaline and the mixture left overnight. It was then filtered and the filtrate acidified with dilute hydrochloric acid in the cold. The acid separated in a semi-solid state which after some time set to a crystalline mass. This was collected, washed and crystallised from dilute alcohol in colourless needles, m.p. 240°. (Found: N, 7.08. $C_{13}H_{11}O_6N_2ClS_2$ requires N, 7.17 per cent).

4-Sulphonyl-(p'-sulphonamidophenyl)-amino-4'-methoxydiphenylamine-2-carboxylic Acid.—2-Chloro-5-sulphonyl-(*p'*-sulphonamidophenyl)-amino-benzoic acid (15.6 g.), *p*-anisidine (5 g.) and anhydrous potassium carbonate (5.5 g.) were refluxed together in amyl alcoholic solution with little copper powder for about 4 hours. The mixture was diluted with a little water and distilled in steam. The residue was then filtered and the filtrate acidified in the cold with dilute hydrochloric acid. The acid, thus obtained, was crystallised from dilute alcohol (charcoal) in almost colourless needles, m.p. 246°. (Found: N, 8.45. $C_{20}H_{19}O_7N_3S_2$ requires N, 8.8 per cent).

3-Sulphonyl-(p'-sulphonamidophenyl)-amino-7-methoxy-5-chloroacridine.—4-Sulphonyl-(*p'*-sulphonamidophenyl)-amino-4'-methoxydiphenylamine-2-carboxylic acid was heated with excess of phosphorus oxychloride on a boiling water-bath for more than 2 hours and then the oxychloride was removed in *vacuo*. The residual mass was decomposed with ice-cold water and made just alkaline with ammonia. The solid obtained was crystallised from a mixture of acetone and water (charcoal) in brownish yellow needles, m.p. 212–15°. The compound is soluble in dilute alkalis and acids and is slightly fluorescent in alcoholic solution. (Found: N, 8.82; Cl, 7.28. $C_{20}H_{18}O_5N_3ClS_2$ requires N, 8.79; Cl, 7.43 per cent).

3-Sulphonyl-(p'-sulphonamidophenyl)-amino-7-methoxy-5-(ω -diethylaminoisopropyl)-aminoacridine (structure analogous to II).—To a solution of 3-sulphonyl-(*p'*-sulphonamidophenyl)-amino-7-methoxy-5-chloroacridine (3 g.) dissolved in phenol (10 g.) at 100° δ -diethylamino- α -methylbutylamine (2 g.) was added slowly and the solution kept at 100° for 2 hours. It was then poured into about 150 c.c. of 5% sodium hydroxide solution in the cold. The alkaline solution was extracted several times with ether and made almost neutral by adding hydrochloric acid when a semi-solid mass separated. It was collected, washed with ice-cold water and dissolved in glacial acetic acid

and filtered. The acetic acid solution was extracted several times with ether and made strongly alkaline with ammonia in the cold, and left overnight. The precipitate obtained was washed, dried and crystallised from acetone-petroleum ether mixture (1 : 4) in yellow crystals, m.p. 254-56°. (Found: N, 11.82; S, 10.9. $C_{20}H_{17}O_5N_3S_2$ requires N, 11.69; S, 10.68 per cent). The compound is tasteless and soluble in acids and alkalis. It gives high fluorescence (emerald green) in alcoholic solution; the acid and alkali solutions give weaker fluorescence, showing that it exists in acridonimine form (Basu and Das-Gupta, *loc. cit.*).

3-Sulphonyl-(*p'*-sulphonamidophenyl)-amino-7-methoxy-5-(δ -diethylaminobutyl)-aminoacridine (structure analogous to II).—3-Sulphonyl-(*p'*-sulphonamidophenyl)-amino-7-methoxy-5-chloroacridine (3 g.) dissolved in phenol (10 g) at 100° was condensed with δ -diethylaminobutylamine (2 g.) for 2 hours. The acridine derivative was isolated as in the foregoing compound and crystallised from acetone-petroleum ether mixture in yellow crystals, m.p. 220-22°. Its properties are similar to the previous compound. (Found: N, 11.65; $C_{28}H_{35}O_5N_5S_2$ requires N, 11.96 per cent).

2-Chloro-5-sulphonyl-(*p'*-sulphondiethylamidophenyl)-aminobenzoic Acid.—*p*-Aminobenzenesulphon-diethylamide (2 mol.) and 1-carboxy-2-chlorobenzene-5-sulphonyl chloride (1 mol.) were reacted in the manner as in the previous case. The acid, isolated as a semi-solid mass, set to crystalline solid on treatment with ether. It separated from dilute alcohol in colourless needles, m.p. 194-95°. (Found: N, 6.15. $C_{17}H_{19}O_6N_2ClS_2$ requires N, 6.27 per cent).

4-Sulphon-(*p'*-sulphondiethylamidophenyl)-amino-4'-methoxydiphenylamine-2-carboxylic Acid.—2-Chloro-5-sulphonyl-(*p'*-sulphondiethylamidophenyl)-aminobenzoic acid and *p*-anisidine were condensed in amyl alcoholic solution with a little copper powder and anhydrous potassium carbonate for 4 hours. After usual treatment, the diphenylamine acid was crystallised from dilute alcohol (charcoal) in almost colourless needles, m. p. 202-3°. (Found: N, 7.67. $C_{24}H_{27}O_7N_3S_2$ requires N, 7.88 per cent).

3-Sulphonyl-(*p'*-sulphondiethylamidophenyl)-amino-7-methoxy-5-chloroacridine. — 4-Sulphon-(*p'*-sulphondiethylamidophenyl)-amino-4'-methoxydiphenylamine-2-carboxylic acid was acridinated with phosphorus oxychloride at 100° on a water-bath for 2 hours. The chloroacridine was isolated as usual and crystallised from acetone in yellow needles, m.p. 187-89°. It gave weak fluorescence in alcoholic solution. (Found: N, 7.92; Cl, 6.42. $C_{24}H_{24}O_5N_3ClS_2$ requires N, 7.87, Cl, 6.65 per cent).

3-Sulphonyl-(*p*-sulphondiethylamidophenyl)-amino-7-methoxy-5-(ω -diethylaminoisoamyl)-aminoacridine.—The above chloroacridine (3 g.)

dissolved in phenol (10 g.) was condensed with δ -diethylamino- α -methylbutylamine (1.6 g.) by heating for 1½ hours. The acridine derivative was finally crystallised in orange-yellow crystals from a mixture of acetone and petroleum ether melting indefinitely at 160°. It is almost tasteless but possesses all the other usual characteristics. (Found: N, 10.65; S, 10.04. $C_{33}H_{45}O_5N_5S_2$ requires N, 10.69; S, 9.77 per cent).

3-Sulphonyl-(p'-sulphondiethylaminophenyl)-amino-7-methoxy-5-(δ -diethylaminobutyl)-aminoacridine.—This was prepared from the corresponding 5-chloroacridine (3 g.) and δ -diethylaminobutylamine (1.5 g.) in the usual way, and was finally obtained in orange-yellow crystals from acetone-petroleum ether, m.p. 130° (indefinite). Its properties are similar to its analogues described. (Found: N, 10.61; S, 9.84. $C_{32}H_{43}O_5N_5S_2$ requires N, 10.91; S, 9.98 per cent).

3-Sulphonyl-(p'-sulphondiethylamidophenyl)-amino-7-methoxy-5-(γ -diethylaminopropyl)-aminoacridine.—This was obtained from the chloroacridine (3 g.) and γ -diethylaminopropylamine (1.4 g.) After isolation in the usual way it crystallised from acetone-petroleum ether in yellow crystals, m.p. 200° (indefinite). (Found: N, 11.27. $C_{31}H_{41}O_5N_5S_2$ requires N, 11.16 per cent).

p-Aminobenzenesulphonylaminobenzene-4-sulphondiethylamide (I, R = Et).—*p*-Aminobenzene-sulphondiethylamide (1 mol.) and *p*-acetylaminobenzenesulphonyl chloride (1 mol.) were rubbed with a little water in a mortar for several hours and left overnight. Sodium carbonate was added occasionally to keep the mixture alkaline. The solid obtained after filtration was extracted with hot benzene. The residue (acetyl derivative) was crystallised from alcohol in colourless crystals, m.p. 228°. (Found: N, 10.03. $C_{18}H_{23}O_3N_3S_2$ requires N, 9.88 per cent).

The acetyl derivative was hydrolysed by refluxing with a little excess of 5% aqueous-alcoholic hydrochloric acid for ¼ hour till a clear solution resulted. This was then made just alkaline with dilute ammonia in the cold. The precipitated solids were collected and crystallised from dilute alcohol in colourless crystals, m.p. 176°. (Found: N, 11.14. $C_{18}H_{21}O_4N_3S_2$ requires N, 10.96 per cent).

Formation of (III, R=Et).—Equimolecular quantities of 2-chloro-7-methoxy-5-chloroacridine and (I, R=Et) were heated in phenol at 140° for about 3 hours. The mixture was then poured into dilute caustic soda solution in the cold. The separated solids were collected and treated with dilute acetic acid and filtered. The residue was dried and crystallised from acetone-petroleum ether mixture (1:4) in bright yellow crystals, m.p. 160–61°. It

gives weak fluorescence in alcoholic solution. (Found : N, 8.66 ; S, 10.14. $C_{30}H_{20}O_5N_4ClS_2$ requires N, 8.96, S, 10.24 per cent).

Formation of (III, R=Et and Me in place of methoxyl).—This was obtained from 2-chloro-7-methyl-5-chloroacridine and I (R=Et) as previously described. It was crystallised from acetone-petroleum ether in yellow crystals, m.p. 133-34°. (Found : N, 9.05. $C_{30}H_{20}O_4N_4ClS_2$ requires N, 9.2 per cent).

The author wishes to express his grateful thanks to Dr. U. P. Basu for his guidance and advice during this investigation.

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Received June 26, 1939.

REVIEW

The Chemistry of Milk—By W. S. DAVIS. PUBLISHED BY CHAPMAN & HALL, 1939 ; DEMY, PP. xiv + 534. PRICE 25 SH. NET.

The volume is the revised second edition of the book which first appeared in 1935 as one of a series of monographs on Applied Chemistry published under the editorship of Dr. E. Howard Tripp. The book is divided into five parts. Part I deals with the composition of milk in all its variations. Part II deals in detail with the different constituents of milk, butter-fat, lactose, proteins, enzymes and the minor constituents of milk. The physico-chemical properties of milk including the coagulation of milk by different agents are treated in Part III. The chemistry of milk processing is dealt with in Part IV, which includes important chapters on the technology of milk condensing and dried milk products. Part V deals with milk in its nutritional aspects. There is an up-to-date bibliography at the end of each chapter, and the book is complete with a subjects' and an authors' index. The book is well printed and well got-up.

The special feature of the book is that it deals comprehensively with the chemistry of milk in practically all its aspects and at the same time the production is very well-balanced and extremely readable. The wealth of matter has not disturbed the sequence of treatment or the natural development of the subject from chapter to chapter in each part of the book. The subject of milk is of obvious importance to chemists, biochemists, physiologists, agriculturists, people in the dairy industry and medical people generally, for all of whom the book supplies latest information in a full and critical manner. Being himself actively associated with dairy research, Dr. Davis has many problems to offer for solution and the mind of the reader is frequently stimulated with reference to such unsolved problems. It is indeed curious to think that although milk is one of the best known articles of diet to mankind, it is only during recent years that many details about some of its nutritive constituents have become known, and it seems probable that many other facts await discovery.

The technological portion of the book deals with the dairy industry in connection with the making of condensed milk, dried milk preparations, etc. Industrial applications of selected milk constituents like, for instance, casein, in the different arts are not dealt with, as they are not apparently included in the scope of the volume.

The book forms a valuable basis on which further work can be done and it can be recommended to practically all people who are interested in one or other aspect of milk. The importance of such monographs in these days of multiplicity of journals and papers is obvious.

B.C.G.

VISCOSITY OF AQUEOUS SOLUTIONS OF FORMIC, CYANOACETIC AND OXALIC ACIDS.

BY M. K. SRINIVASAN AND B. PRASAD.

Viscosities of aqueous solutions of formic, cyanoacetic and oxalic acids have been measured at 35°. The viscosity of these solutions is represented quite well by either of the two equations proposed by the authors, but the values of viscosity constants are not of the expected order. The results are also represented by the Jones and Dole equation, but the constants have no theoretical significance for weak electrolytes.

The authors derived an equation,

$$\frac{\eta - \eta_0}{\eta_0} = A\sqrt{\alpha c} + \beta(1 - \alpha)c \quad \dots (1)$$

connecting viscosity, concentration 'c' and the degree of dissociation 'α' of an aqueous solution of a weak electrolyte, on the assumption that the increase in relative viscosity $\frac{\eta - \eta_0}{\eta_0}$ of such a solution was due to two additive factors, one

$$\frac{\Delta_1}{\eta_0} = A\sqrt{\alpha c} + B\alpha c,$$

due to the dissociated part (electrolyte) and the other,

$$\frac{\Delta_2}{\eta_0} = \beta(1 - \alpha)c$$

due to the undissociated part (non-electrolyte) (*Trans. Faraday Soc.*, 1938, **34**, 1139). In the case of dilute solutions of very weak electrolytes the term 'B α c' could be neglected as the value of 'α' and 'c' are very small. The equations represented the results satisfactorily in the case of very weak electrolytes (*loc. cit.*), but the experimental values of 'A' did not agree with the theoretical value (Falkenhagen and Vernon, *Phil. Mag.*, 1932, **14**, 537).

It was considered desirable to extend the work to a few more weak electrolytes for some of which the term 'B α c' could be taken into account due to their fairly large degree of dissociation. In such cases the equation would assume the form,

$$\frac{\eta - \eta_0}{\eta_0} = A\sqrt{\alpha c} + B\alpha c + \beta(1 - \alpha)c \quad \dots (2)$$

With this object the viscosities of dilute solutions of formic, cyanoacetic and oxalic acids have been measured.

EXPERIMENTAL.

Measurements were made at $35^{\circ} \pm 0.005^{\circ}$ in a thermostat described in the previous communication (*loc. cit.*). Particulars about the viscometer used are given below.

Approx. time of flow for water	= 2141 sec.
Approx. capacity of bulb on the capillary side	= 7.5 c.c.
Length of capillary tube	= 10.5 cm.
Diameter of the capillary bore	= 0.28 mm.

No kinetic energy correction was found to be necessary. On account of the design of the viscometer, surface tension correction was eliminated (*Trans. Faraday Soc.*, 1939, **35**, 374). The time of flow was measured to an accuracy of 0.2 second by means of a Zenith stop-watch for formic and cyanoacetic acids and by a Venner time-switch (marked off in tenths of seconds) for oxalic acid. Density measurements were made with two pyknometers of approximate capacity of 20 c.c. and 63 c.c., the smaller one for formic and cyanoacetic acid and the larger for oxalic acid. The maximum error in viscosity measurements, calculated on the basis of an error of 0.2 second in measuring time of flow and 0.001 g. in determining the mass of the pyknometer, works to 0.0002. The mean of three to five readings for time and two readings for mass were taken for calculating the viscosity.

Kahlbaum's formic acid was redistilled before use. Solutions of oxalic acid were made from Kahlbaum's 'pro analysi' sample after crystallisation. Cyanoacetic acid (Merck's quality marked for scientific purposes) of m.p. 64° was used as such.

The values of the dissociation constants, ' K ' were taken from the Landolt-Bornstein Tabellen. The constants in equation (1) were evaluated graphically by plotting $\frac{\eta}{\eta_0} - 1 / \sqrt{\alpha c}$ against $(1 - \alpha) \sqrt{c} / \alpha$, the slope of the straight line giving the value of ' β ' and the intercept at the ordinate, the value of A . The constants in equation (2) were obtained by solving simultaneous equations. In the following tables concentration in g. mols. per litre, density in g./c.c., relative viscosity observed $\frac{\eta}{\eta_0}(0)$, and viscosity calculated by equation (1), $\frac{\eta}{\eta_0}(1)$ and equation (2), $\frac{\eta}{\eta_0}(2)$ are given,

TABLE I.

Formic acid.

$$K = 2.4 \times 10^{-4}, \quad A = 0.00, \quad \beta = 0.048.$$

Conc.	Density.	$\frac{\eta}{\eta_0}$ (0).	$\frac{\eta}{\eta_0}$ (1)	Conc.	Density.	$\frac{\eta}{\eta_0}$ (0).
0	0.99406					
0.01086	0.99440	1.0007	1.0005	0.21842	0.99535	1.0084
0.02184	0.99448	1.0011	1.0009	0.32763	0.99548	1.0124
0.03271	0.99451	1.0013	1.0014	0.45584	0.99898	1.0164
0.04361	0.99463	1.0018	1.0020	0.56980	0.99984	1.0195
0.05452	0.99467	1.0021	1.0025	0.68376	1.00133	1.0236
0.06542	0.99477	1.0029	1.0030	0.79772	1.00255	1.0276
0.07632	0.99503	1.0037	1.0035	0.91168	1.00371	1.0310
0.08722	0.99512	1.0042	1.0040	1.02560	1.00482	1.0347
0.09813	0.99524	1.0047	1.0045	1.13960	1.00616	1.0381
0.10921	0.99531	1.0048	1.0050			

TABLE II.

Cyanoacetic acid.

$$K = 3.7 \times 10^{-3}; \quad (1) \quad A = 0.018, \quad \beta = 0.13.$$

$$(2) \quad A = 0.00, \quad B = 0.172, \quad \beta = 0.122.$$

Conc.	Density.	$\frac{\eta}{\eta_0}$ (0).	$\frac{\eta}{\eta_0}$ (1).	$\frac{\eta}{\eta_0}$ (2).
0.00800	0.99417	1.0014	1.0010	1.0012
0.02500	0.99463	1.0037	1.0038	1.0035
0.03500	0.99467	1.0047	1.0050	1.0048
0.04500	0.99496	1.0063	1.0063	1.0061
0.05594	0.99537	1.0075	1.0077	1.0074
0.06500	0.99564	1.0088	1.0088	1.0086
0.07513	0.99580	1.0095	1.0100	1.0099
0.08500	0.99609	1.0110	1.0112	1.0112
0.10000	0.99643	1.0131	1.0131	1.0131
0.24903	0.99946	1.0282		
0.40847	1.00256	1.0442		
0.70306	1.00824	1.0673		
1.16350	1.01712	1.1242		

TABLE III.

Oxalic acid. $K = 3.8 \times 10^{-2}$. (1) $A = 0.022$, $\beta = 0.31$ (2) $A = 0.025$, $B = -0.024$, $\beta = 0.33$.

Conc.	Density.	$\frac{\eta}{\eta_0}$ (0)	$\frac{\eta}{\eta_0}$ (1).	$\frac{\eta}{\eta_0}$ (2).
0.005013	0.99426	1.0016	1.0016	1.0017
0.010026	0.99450	1.0028	1.0026	1.0027
0.015039	0.99474	1.0034	1.0035	1.0036
0.020052	0.99497	1.0047	1.0045	1.0046
0.030078	0.99539	1.0062	1.0065	1.0064
0.035091	0.99552	1.0073	1.0073	1.0075
0.040104	0.99585	1.0085	1.0083	1.0085
0.045117	0.99604	1.0098	1.0094	1.0096
0.057843	0.99653	1.0122	1.0121	1.0123

DISCUSSION.

As seen from the foregoing tables, the results are represented satisfactorily in all cases. Equation (2) was not applied to formic acid as the values ' αc ' are small. The equations fail in the sense that the experimental values of A do not agree with the theoretical value, i.e., about 0.003. In view of this wide difference the authors propose to test the correctness of the fundamental assumption (simple additivity of the viscosity of dissociated and undissociated parts) by measuring the viscosity of solutions of dextrose and some electrolytes, separately and together.

The Jones and Dole equation (*J. Amer. Chem. Soc.*, 1929, **51**, 2950) represents the results in all cases but the values of A could have no theoretical significance for weak electrolytes.

One of us (M. K. S.) is grateful to the Government of Orissa for the grant of a research scholarship.

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Received May 18, 1939.

PERIODATES OF YTTRIUM, ERBIUM AND CERIUM.

By R. K. BAHL AND SURJIT SINGH.

Yttrium, cerium and erbium resemble with the rest of the rare earths in forming the corresponding rare earth mesoperiodate YIO_5 usually with four molecules of water. Yttrium differs from the rest in forming an additional periodate, yttrium diorthoperiodate with eleven molecules of water, $\text{Y}_4\text{I}_2\text{O}_{13}$, $11\text{H}_2\text{O}$, or $2\text{Y}_2\text{O}_3$, I_2O_7 , $11\text{H}_2\text{O}$.

Cleve found that yttrium acetate gives two periodates (*Bull. Soc. chim.*, 1874, 21, 196) $3\text{Y}_2\text{O}_3 \cdot 2\text{I}_2\text{O}_7$, $6\text{H}_2\text{O}$ and YIO_5 , $4\text{H}_2\text{O}$. The latter was obtained by dissolving the precipitate formed by the interaction of paraperiodic acid and then recrystallising it out.

On repeating the above work, yttrium acetate was found to give only one periodate on its treatment with paraperiodic acid, namely the tetrahydrated yttrium mesoperiodate, $\text{YIO}_5 \cdot 4\text{H}_2\text{O}$.

A new yttrium periodate, i.e., yttrium diorthoperiodate with eleven molecules of water, $\text{Y}_4\text{I}_2\text{O}_{13}$, $11\text{H}_2\text{O}$ or $2\text{Y}_2\text{O}_3$, I_2O_7 , $11\text{H}_2\text{O}$ (described later) has also been obtained.

Tetrahydrated mesoperiodates were obtained in the case of erbium and cerium.

EXPERIMENTAL.

General Method of Preparation of the Periodates of Yttrium, Erbium and Cerium.

The preparation of the periodates of yttrium, erbium and cerium was carried out by the gradual addition of a dilute solution of paraperiodic acid to a cold solution of a soluble rare earth salt or by boiling a suspension of disodium paraperiodate with a solution of an excess of the soluble rare earth salt. In the latter case the mixture was boiled for half an hour to ensure complete reaction. The precipitate obtained in each case was filtered, washed and dried at 40° in an electric air oven.

The earth elements were estimated by igniting the periodates to oxides. Iodine and available oxygen were determined by the methods adopted by Partington and Bahl (*J. Chem. Soc.*, 1934, 1085, 1087).

Periodates of Yttrium.

(a) A new salt, yttrium diorthoperiodate with eleven molecules of water, $Y_4I_2O_{13} \cdot 11H_2O$ or $2Y_2O_3 \cdot I_2O_7 \cdot 11H_2O$ was formed by the interaction of disodium paraperiodate and yttrium acetate, in the form of a white precipitate; it formed a white amorphous powder in the dry state.

TABLE I.

Sample.	Yttrium		Iodine		Available oxygen	
	Found.	Calc.	Found.	Calc.	Found.	Calc.
1	35.44%	35.0%	25.20%	25.03%	10.67%	11.03%
2	35.01		25.13		10.42	
3	35.20		25.40		10.60	

Yttrium mesoperiodate with four molecules of water, $YIO_5 \cdot 4H_2O$ was obtained by the action of paraperiodic acid on yttrium acetate. This salt, which is in the form of a fine precipitate, dissolves in excess of paraperiodic acid and on crystallisation from this solution yields the same mesoperiodate (see A). We have been unable to prepare the salt $3Y_2O_3 \cdot 2I_2O_7 \cdot 6H_2O$ as claimed by Cleve (*loc. cit.*).

TABLE II.

Sample A is the recrystallised salt.

Sample	Yttrium		Iodine		Available oxygen	
	Found.	Calc.	Found.	Calc.	Found.	Calc.
1	24.47%	24.12%	33.50%	34.54%	15.63%	15.23%
2	24.20		33.20		15.27	
3	24.22		33.54		15.30	
A	24.16		34.13		15.30	

Periodate of Erbium.

(b) *Tetrahydrated Erbium Mesoperiodate*, $\text{ErIO}_5 \cdot 4\text{H}_2\text{O}$, was formed as a white gelatinous precipitate by boiling a solution of erbium nitrate with a suspension of disodium paraperiodate. It is a white powder in the dry state.

TABLE III.

Sample.	E r b i u m		I o d i n e		Available oxygen	
	Found.	Calc.	Found.	Calc.	Found.	Calc.
1	36.10%	37.54%	28.60%	28.42%	12.60%	12.5%
2	36.30		29.31		12.90	
3	36.40		28.94		12.70	
4	36.30		29.00		12.80	

The slight discrepancies in the results of erbium and iodine are probably due to the presence of traces of other rare earths present in the erbium nitrate, which are very difficult to eliminate.

No precipitate was obtained by the addition of paraperiodic acid to cold or hot solution of erbium nitrate.

Periodate of Cerium.

(I) *Tetrahydrated Cerium Mesoperiodate*, $\text{CeIO}_5 \cdot 4\text{H}_2\text{O}$.—A yellow precipitate was obtained by the addition of a suspension of disodium paraperiodate to a boiling solution of cerium nitrate. It turned deep yellow on drying.

(II) The same salt was also obtained by the action of paraperiodic acid on cerium nitrate. In this case a white gelatinous precipitate, first formed, turned yellow on standing. We confirm this observation made by Cleve (*loc. cit.*).

The results given in Table IV show that the yttrium, erbium and cerium rare earths resemble each other in the formation of the same periodate, namely tetrahydrated rare earth mesoperiodate.

TABLE IV.

Samples A, prepared by method (I).

Samples B, prepared by method (II).

Sample.	Cerium		Iodine		Available oxygen	
	Found.	Calc.	Found	Calc	Found.	Calc.
A 1	33.70%	33.40%	29.82%	30.20%	11.60%	11.45%
2	33.61		30.27		11.61	
3	33.41		29.77		11.40	
B 1	33.10		29.86		11.53	
2	33.42		30.41		11.44	
3	33.51		30.12		11.44	

This corresponds with the results obtained in case of lanthanum and samarium (*Bull. Soc. chim.*, 1874, **21**, 196; 1883, **39**, 289). These form dihydrated lanthanum mesoperiodate $\text{LaIO}_5 \cdot 2\text{H}_2\text{O}$ and tetrahydrated samarium mesoperiodate $\text{SmIO}_5 \cdot 4\text{H}_2\text{O}$ respectively. This confirms a close similarity between the different salts of the rare earths. Yttrium, however, differs from the rest in the formation of one more periodate, $\text{Y}_4\text{I}_2\text{O}_{13} \cdot 11\text{H}_2\text{O}$ or $2\text{Y}_2\text{O}_3 \cdot \text{I}_2\text{O}_7 \cdot 11\text{H}_2\text{O}$.

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Received July 24, 1939.

THE HYDROGEN ION CONCENTRATION OF SOLUTIONS CONTAINING ZINC HYDROXIDE AND SODIUM HYDROXIDE.

By S. M. MEHTA AND M. B. KABADI.

In continuation of the work described before the hydrogen ion concentration of solutions containing ZnO and Na₂O in varying proportions has been determined using the glass electrode. The results obtained previously are confirmed and it is inferred that in solutions more dilute than 6*N*, the sodium zincate is largely hydrolysed, whereas in concentrated solutions the hydrolysis is small. The possibility of isolating sodium zincate from solutions stronger than 8*N* has been suggested.

The data obtained by measuring the electrical conductivity of solutions containing zinc hydroxide and sodium hydroxide have been discussed in a previous communication (*J. Indian Chem. Soc.*, 1939, **16**, 223). In order to get further information regarding these solutions, the determination of hydrogen ion concentration was undertaken as it affords an important method for the study of acid-base equilibria. The usefulness of the method in the study of the zincate problem has been already recognised.

Kunschert (*Z. anorg. Chem.*, 1904, **41**, 337), who made a potentiometric study of alkaline solutions containing zinc hydroxide, inferred that in these solutions zinc is present chiefly in the form of ZnO₂ ions, which are partly hydrolysed into HZnO₂ and OH ions. Fricke and Ahrndts (*ibid.*, 1924, **134**, 344) made similar measurements using mercuric oxide electrode and came to the conclusion that an alkali zincate is formed in strongly alkaline solutions. On the other hand, Hildebrand and Bowers (*J. Amer. Chem. Soc.*, 1916, **38**, 785) titrated potentiometrically solutions of zinc halides by means of an alkali and from the curves obtained by plotting the volume of sodium hydroxide against E.M.F. suggested that (i) weak acids of the type HZnCl₃ exist in solution, and (ii) a solution of zinc hydroxide in sodium hydroxide behaves both as a colloid and as a mono-basic acid.

Britton (*J. Chem. Soc.*, 1925, 2120) examined the behaviour of zinc hydroxide during its precipitation from a solution of zinc sulphate by means of sodium hydroxide. He observed that zinc hydroxide was precipitated at a p_H 5.2 and that the solubility product calculated from this value is not in agreement with that already known. He considered that the precipitated zinc hydroxide contained no basic

salt which was formed during the titration and that there was no indication of the acidic nature of the hydroxide when alkali was added to redissolve the precipitate. Britton and Robinson (*Trans. Faraday Soc.*, 1932, **28**, 540) used the glass electrode in the study of this problem and observed that the precipitation of zinc hydroxide from a solution of zinc sulphate takes place at a somewhat lower p_H than from the corresponding solution of zinc chloride. They were, however, unable to reproduce the p_H curves obtained by Kolthoff and Kameda (*J. Amer. Chem. Soc.*, 1931, **53**, 832) and by Prytz (*Z. anorg. Chem.*, 1931, **200**, 133).

EXPERIMENTAL.

The hydrogen ion concentration of solutions prepared as described in the previous communication (*loc. cit.*) and containing the different amounts of zinc hydroxide and sodium hydroxide (expressed as the ratio $ZnO : Na_2O$) was measured using the glass electrode. The valve unit required for the measurement of the E.M.F. of the glass electrode system was constructed according to the details given by Mehta (*J. Univ. Bombay*, 1936, **5**, ii, 77). The apparatus was standardised by means of buffer solutions of known p_H . Two such solutions, *viz.*, $M/20$ -potassium hydrogen phthalate (p_H 3.97) and $M/20$ -borax (p_H 9.15) were used. The former was prepared according to the method of Dodge (*Ind. Eng. Chem.*, 1915, **7**, 29; *J. Amer. Chem. Soc.*, 1920, **42**, 1655) and the latter was Merck's G. R. quality.

The Morton glass electrode cell was placed in an electrically heated thermostat which was zinc-lined and earthed. Its temperature was maintained constant at $30^\circ \pm 0.5^\circ$. The E.M.F. of the combination

Pt	Normal HCl saturated with quin- hydrone.	Glass membrane	Test solution	KCl saturated	KCl saturated calomel	Hg
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was measured with the help of the valve unit referred to above. From the observed E.M.F. the p_H was calculated by means of the equation,

$$p_H = \frac{0.4535 - E}{0.06}.$$

The glass electrode was checked immediately before and after each set of readings. The p_H values calculated from the observed E.M.F. and checked by means of the calibration curve are given in the following table in which N denotes concentration of the solution in normality of its sodium hydroxide content,

TABLE I.

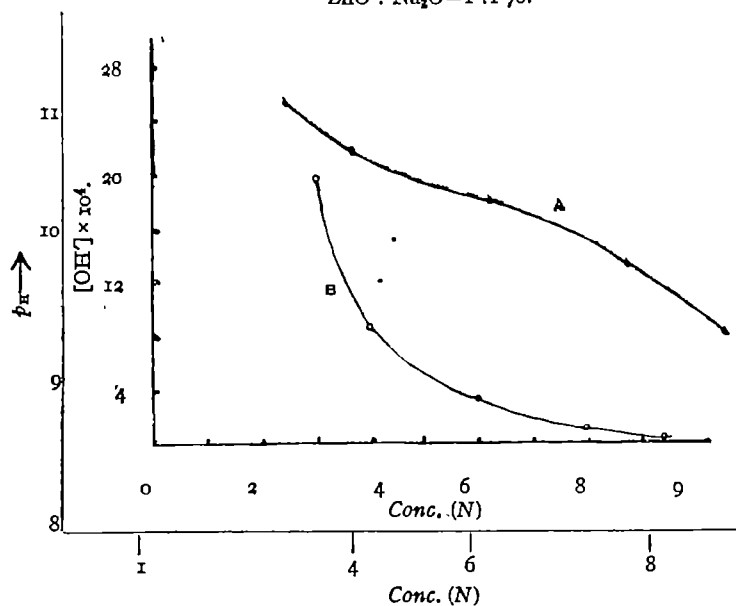
N.	Ratios of ZnO:Na ₂ O				
	1:1.76.	1:2.44.	1:3.03.	1:3.55.	1:4.05.
9.41	9.297	—	—	—	—
9.20	—	9.375	—	—	—
9.50	—	—	9.275	—	—
9.48	—	—	—	9.417	—
9.47	—	—	—	—	9.433
8	9.783	9.625	9.668	9.762	9.808
6	10.262	10.142	10.195	10.272	10.375
4	10.653	10.546	10.495	10.643	10.638
3	11.015	10.763	10.742	10.855	10.862
2	—	10.992	10.880	11.027	11.017
1.5	—	11.083	11.012	11.170	11.200
1	—	—	11.200	11.302	11.362

DISCUSSION.

It will be noticed from the table that the p_H of all the solutions used in this investigation increases with dilution and that it lies between 9 and 12. When the p_H is plotted against concentration, curves are obtained (only one illustrative curve is shown in Fig. 1A) which show that the p_H increases at first rapidly, then slowly for some time and finally rapidly once again as the dilution is increased.

FIG. 1.

ZnO:Na₂O=1:1.76.



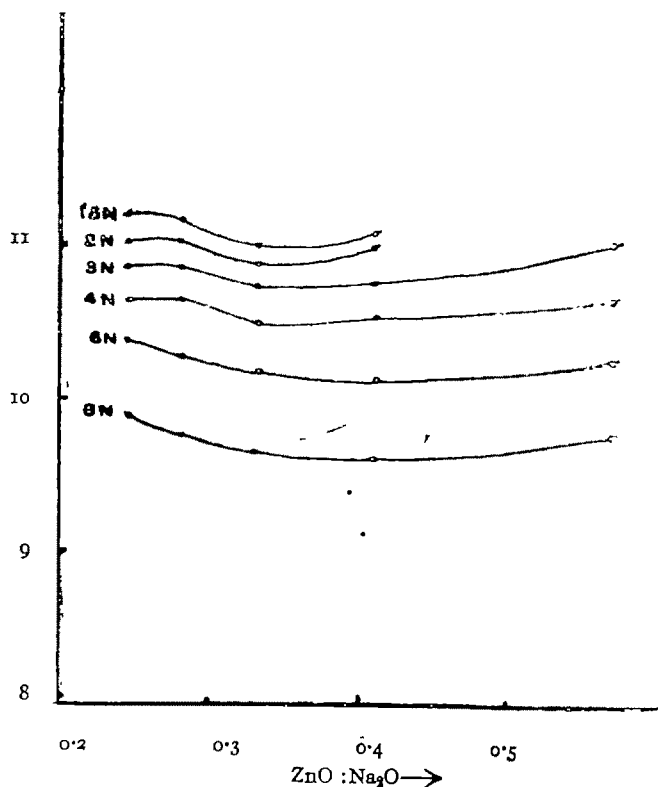
If, however, the hydroxyl ion concentration calculated by means of the equation

$$\log \frac{1}{[\text{OH}']} - pK_w - p_H = 13.725 - p_H$$

is plotted against concentration, curves of the type illustrated in Fig. 1B are obtained which show that the hydroxyl ion concentration increases at first slowly and then rapidly with dilution.

The increase in p_H or OH' ion concentration on dilution referred to above appears to be due to an increase in the hydrolysis of sodium zincate present in the concentrated solutions. It was stated in the previous communication (*loc. cit.*) that the increase in the equivalent conductivity of these solutions on dilution is to be attributed to an increase in the number of mobile hydroxyl ions. The data presented in this paper confirm the above statement.

FIG. 2.

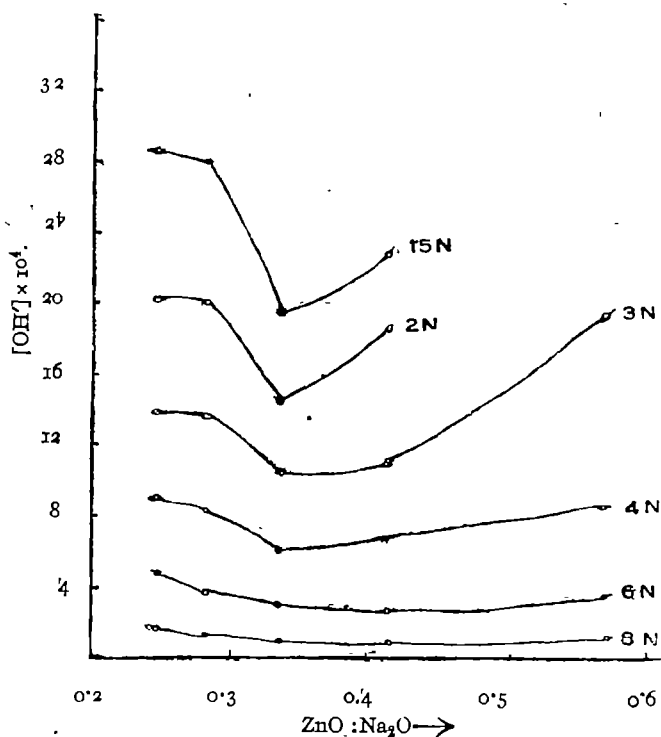


When the p_H is plotted against the ratio $\text{ZnO}:\text{Na}_2\text{O}$, curves are

obtained (*vide* Fig. 2) which are concave towards the ratio axis. These curves are similar to those obtained by plotting the equivalent conductivity against the ratio $\text{ZnO}:\text{Na}_2\text{O}$ described in the previous communication (*loc. cit.*). This fact leads one to infer that the variation in the equivalent conductivity just referred to is to be ascribed to a change in p_H of the solutions. It is clearly seen from the curves that the p_H decreases with an increase in the relative amount of sodium hydroxide, reaches a minimum and then increases again. In other words as the ratio $\text{ZnO}:\text{Na}_2\text{O}$ is decreased, there is at first a decrease in p_H followed by an increase. This behaviour of the solutions may be explained as being due to a diminution in the hydrolysis of sodium zincate on the addition of sodium hydroxide. In accordance with this explanation the minimum points of the different curves correspond to the maximum repression of hydrolysis after which there is an increase in p_H owing to the hydroxyl ions coming from the added alkali.

The variation in hydroxyl ion concentration with a change in the ratio $\text{ZnO}:\text{Na}_2\text{O}$ is represented graphically in Fig. 3 from which it is seen

FIG. 3.



that the nature of these curves is similar to that of the curves in Fig. 2 with this difference, however, that the variation in OH' ion concentration with a change in the ratio $\text{ZnO} : \text{Na}_2\text{O}$ is more pronounced than the variation in p_{H} . It is also seen from Fig. 3 that in solutions, more concentrated than 6N, the OH ion concentration diminishes but little on decreasing the ratio $\text{ZnO} : \text{Na}_2\text{O}$. This behaviour may be due to the fact that in these concentrated solutions the sodium zincate present is hydrolysed only to a small extent and that therefore there is no marked change in OH ion concentration on increasing the relative proportion of sodium hydroxide.

The behaviour of solutions more dilute than 6N is different. The OH ion concentration falls in the beginning, reaches a minimum and then increases again. It seems probable that in dilute solutions sodium zincate is largely hydrolysed and that the relative increase in the amount of sodium hydroxide represses the hydrolysis with a consequential decrease in the hydroxyl ion concentration until the minimum value is reached. After this point further increase in the relative proportion of sodium hydroxide has very little effect on the hydrolysis of sodium zincate and the OH ion concentration increases due to the hydroxyl ions coming from the alkali.

From the results obtained in this investigation and the conclusions arrived at above it appears that it should be possible to isolate sodium zincate from solutions stronger than 8N. This has been confirmed and the details of the experiments in which sodium zincate was isolated in a state of purity will be published shortly.

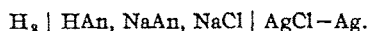
PHYSICAL AND INORGANIC CHEMISTRY DEPARTMENT,
THE ROYAL INSTITUTE OF SCIENCE,
BOMBAY.

Received July 10, 1939.

DISSOCIATION CONSTANT OF β -RESORCYLIC ACID.

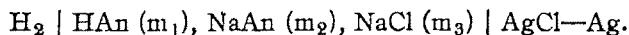
By C. T. ABICHANDANI AND S. K. K. JATKAR.

The dissociation constant K_1^a of β -resorcylic acid has been determined by the measurement of the E. M. F. of the cell without any liquid junction



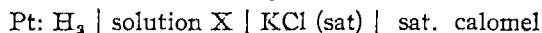
The corrected value of K_1^a in pure water is found to be 6.05×10^{-4} at 30° .

In the course of a systematic research on the dissociation constants of hydroxybenzoic acids (*J. Indian Inst. Sci.*, 1938, **21A**, 417) we have shown that the order of the first dissociation constants (K_1^a) is *ortho* > *meta* > *H* > *para*, while that of the second dissociation constants (K_2^a), for which there are no previous values in literature, is the reverse. The first dissociation constant of the gallic acid is appreciably less than that of benzoic acid, which is surprising in view of the fact that the OH groups, due to the electron attracting di-pole, should increase the strength of the acid. It was, therefore, interesting to study the first dissociation constants of α -, β - and γ -resorcylic acids. The previous results of the dissociation constants for the resorcylic acids were determined by the conductivity method (*cf.* Ostwald, *Z. physikal. Chem.*, 1889, **3**, 249; Süss, *Monatsh.*, 1905, **26**, 1331) and were not corrected for the effect of interionic attraction. In the present paper we have presented the results obtained for the dissociation constants of β -resorcylic acid by measuring the E.M.F. of the cells



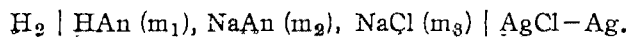
The value of K_1^a is 6.05×10^{-4} as compared with K_1^a 5.16×10^{-4} and 4.96×10^{-4} of Ostwald and Süss respectively.

In the previous paper we had determined the dissociation constants by the method of potentiometric titrations after measuring the p_H at various stages of the titration. The p_H at any point was computed from the E.M.F. of the cell



The junction potential between the solution X and saturated calomel was assumed to be small and neglected which really is not the case (*J. Bureau Standards*, 1936, **16**, 575). It was, therefore, necessary to use a method in which there will be no uncertainty regarding the junction potential.

The use of silver—silver chloride electrode for the determination of dissociation constants of weak acids has been described by Harned and co-workers (*J. Amer. Chem. Soc.*, 1930, **52**, 5079, 1932, **54**, 1350). The method consists of measuring the electromotive force of the cells of the type

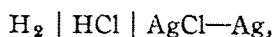


The dissociation constant K is calculated according to the equation

$$E - E_o + \frac{RT}{F} \ln \frac{m_{\text{HAN}} m_{\text{Cl}}}{m_{\text{AN}}} = - \frac{RT}{F} \ln K'$$

$$= - \frac{RT}{F} \ln \frac{\gamma_{\text{HAN}} \gamma_{\text{Cl}}}{\gamma_{\text{AN}}} - \frac{RT}{F} \ln K_1^c$$

where E is the electromotive force of the cell of the above type and the terms represented by m represent the ionic concentrations of the salts in solution. E_o was determined by measuring the E.M.F. of the cell



containing hydrochloric acid of various concentrations. Its value was found to be 0.21920 at 30° in agreement with that reported by

Harned (*loc. cit.*). At zero ionic concentration the term $\ln \frac{\gamma_{\text{HAN}} \gamma_{\text{Cl}}}{\gamma_{\text{AN}}}$ becomes

zero so that K' is equal to K_1^a , the thermodynamical dissociation constant in pure water.

EXPERIMENTAL.

The solutions were prepared from weighed quantities of pure β -resorcylic acid (m. p. 213°) and sodium chloride by adding them to weighed quantities of water. A weighed quantity of standard sodium hydroxide solution (free from carbonate) was run into the solution of the acid and the quantity of sodium salt formed was calculated.

The silver—silver chloride electrode was prepared according to the directions of Carmody (*J. Amer. Chem. Soc.*, 1929, **51**, 2901) and the hydrogen electrode according to the method suggested by Branch, Yabroff and Bettman (*J. Amer. Chem. Soc.*, 1934, **56**, 937). The hydrogen used for the electrode was prepared by the electrolysis of 10% caustic soda and purified in the manner already described (*J. Indian Inst. Sci.*, 1938, **21A**, 373).

The cell (Fig. 1) used in the experiment was made of pyrex glass and was filled with enough solution to dip the hydrogen electrode. For each experiment the electrode was rinsed with solution to be used and the hydrogen was allowed to bubble through the vessel for about 3 hours. The cells were kept in a thermostat maintained at 30° ± 0.02 and the electromotive force readings were taken on a Tinsley's vernier potentiometer.

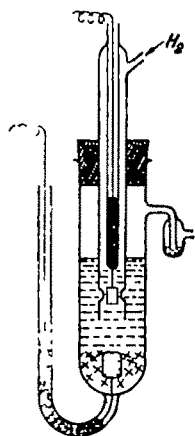


FIG. 1.

The values of the ionic concentrations of various salts along with the observed electromotive force are given in Tables I and II.

TABLE I.

Composition of solutions and E. M. F. of cells.

Solution.	m_{HAc}	m_{NaCl}	m_{NaAc}	μ	E.
I	0.00957	0.03976	0.00864	0.04915	0.5007
II	0.01159	0.03677	0.00773	0.04543	0.4945
III	0.00833	0.02727	0.01137	0.03908	0.5204
IV	0.00755	0.02575	0.00986	0.03648	0.5207
V	0.00961	0.02778	0.00563	0.03415	0.5002
VI	0.00854	0.02184	0.01121	0.03386	0.5242
VII	0.00648	0.01179	0.00466	0.01745	0.5214
VIII	0.00645	0.00563	0.00367	0.01050	0.5344

TABLE II.

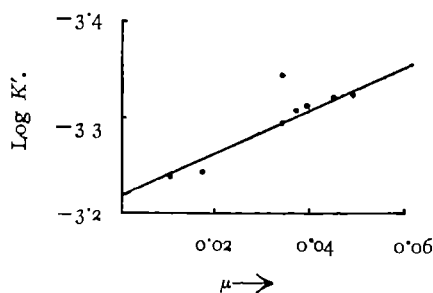
Calculation of dissociation constant.

Solution.	m_{H}	$\frac{E-E_{\infty}}{0.06012}$	$\log \frac{m_{\text{HAc}} m_{\text{Cl}}}{m_{\text{Ac}}}$	$\log K'$	$K' \times 10^4$
I	0.00075	4.6820	-1.3561	-3.3259	4.722
II	0.00093	4.5792	-1.2586	-3.3206	4.779
III	0.00044	5.0100	-1.6993	-3.3107	4.890
IV	0.00087	5.0150	-1.7051	-3.3099	4.899
V	0.00074	4.6740	-1.3320	-3.3420	4.550
VI	0.00081	5.0732	-1.7789	-3.2943	5.078
VII	0.00100	5.0266	-1.7854	-3.2412	5.738
VIII	0.00123	5.2428	-2.0046	-3.2382	5.779

The plot of μ , the ionic strength against the logarithm of the corresponding dissociation constants shown in Fig. 2 when extrapolated for zero

ionic strength gives 6.05×10^{-4} as the value of K_1^a , the true thermodynamical dissociation constant of β -resorcylic acid in pure water at 30° .

FIG. 2.



The lower values obtained by previous workers (Ostwald and Süss *loc. cit.*) are principally due to the then accepted assumption of incomplete dissociation of the sodium salt and to the lack of corrections for the inter-ionic attractions. The two corrections, however, are of opposite sign.

β -Resorcylic acid has two OH groups in *ortho*- and *para*- positions. We have previously shown that the presence of the hydroxyl group in the *ortho*-position increases (the dissociation constant), while that in the *para*- position decreases. In β -resorcylic acid, therefore, the presence of the hydroxyl group in the *ortho*- position increases the strength of the carboxyl acid, while that in the *para*- position decrease it, so that the order of the strength of the acids is in *ortho*-hydroxybenzoic acid > β -resorcylic acid > benzoic acid. Further work is in progress.

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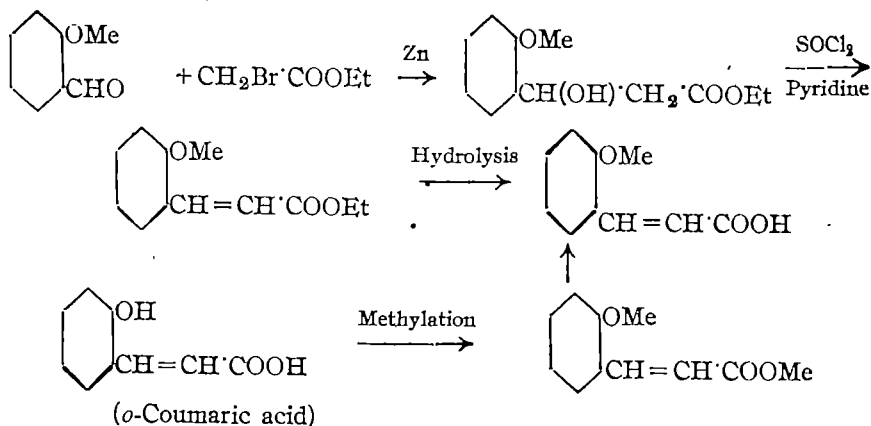
Received August 4, 1939.

SYNTHESIS OF COUMARINS FROM *o*-HYDROXY-ARYL- ALKYL KETONES. PART II. FORMATION OF *o*-COUMARIC ACIDS FROM *o*-HYDROXY- ALDEHYDES.

By DUHKHAHARAN CHAKRAVARTI AND BRAJESWAR MAJUMDAR.

The methyl ethers of the *o*-hydroxy-aldehydes, *e.g.*, 2-methoxybenzaldehyde, 2:4-dimethoxybenzaldehyde, 5-chloro-2-methoxybenzaldehyde, condense with ethyl bromoacetate and ethyl α -bromopropionate forming *trans*-cinnamic esters, which do not yield coumarins.

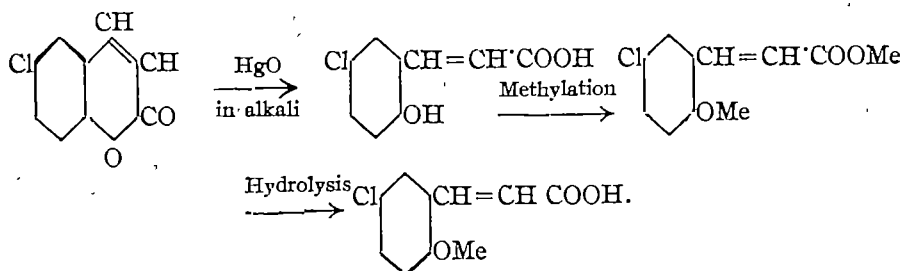
In attempting to synthesise coumarins from *o*-hydroxy-aldehydes by the method described by the authors (Chakravarti and Majumdar, *J. Indian Chem. Soc.*, 1938, 15, 136) it has been found that *trans*-cinnamic esters are formed, which do not yield coumarins on heating with hydriodic acid or on keeping with concentrated sulphuric acid in the cold as in the case of the cinnamic esters, obtained from *o*-hydroxy-aryl-alkyl ketones. Thus 2-methoxybenzaldehyde and 2:4-dimethoxybenzaldehyde readily condense with ethyl α -bromoacetate forming hydroxy-esters, which undergo dehydration with thionyl chloride in the presence of pyridine forming cinnamic esters but the latter do not form the expected coumarins. Ethyl 2-methoxycinnamate, obtained from benzaldehyde, has been shown to be the *trans*-variety as on hydrolysis it forms methoxy-*o*-coumaric acid, identical with the acid prepared from authentic *o*-coumaric acid by methylation and subsequent hydrolysis.



Similarly 2:4-dimethoxybenzaldehyde forms ethyl 2:4-dimethoxycinnamate, which on hydrolysis yields 2:4-dimethoxy-*trans*-cinnamic acid described by Perkin and Schiess (*J. Chem. Soc.*, 1904, 85, 162 ; Tiemann and Will, *Ber.*,

1882, **18**, 2079). 2-Methoxy-benzaldehyde on condensation with ethyl α -bromo-propionate forms ethyl *trans*-2-methoxy- α -methyl cinnamate as it is hydrolysed to *trans*-2-methoxy- α -methyl-cinnamic acid identical with the compound prepared by Perkin (*J. Chem. Soc.*, 1877, **31**, 415).

In order to study the influence of any group in the phenyl nucleus on the formation of *trans*-cinnamic esters, 5-chloro-2-methoxy-benzaldehyde has been condensed with ethyl α -bromoacetate and it has been found that ethyl 5-chloro-2-methoxy-*trans*-cinnamate is formed and the acid obtained after hydrolysis of the ester has been identified to be *trans*-5-chloro-2-methoxycinnamic acid by comparing it with an authentic specimen prepared by the methylation of 5-chloro-*o*-coumaric acid, which has been obtained in quantitative yield by heating an alkaline solution of 6-chlorocoumarin with mercuric oxide (*cf.* Sen and Chakravarti, *J. Indian Chem. Soc.*, 1930, **7**, 247).



The above experimental facts show that if there are no β -alkyl group in the resulting cinnamic ester, the latter has the *trans*-configuration. If the cinnamic esters formed have an alkyl substituent in β -position *cis*-configuration is obtained and these *cis*-*o*-methoxycinnamic esters easily form coumarins. The 2-methoxy-aldehydes, therefore, offer useful starting materials for the synthesis of *o*-coumaric acid derivatives in highly satisfactory yields.

The present paper also describes the synthesis of 7-methoxy-3:4-dimethylcoumarin and 7-hydroxy-3:4-dimethylcoumarin from resacetophenone dimethyl ether and ethyl α -bromopropionate. Resacetophenone dimethyl ether, however, readily forms ethyl 2:4-dimethoxy- α -methylcinnamate with ethyl α -bromoacetate but it does not form coumarin under the usual conditions.

EXPERIMENTAL.

Ethyl 2-Methoxy-trans-cinnamate.—A solution of 2-methoxybenzaldehyde (11 g.) in sodium-dried benzene (50 c.c.), zinc wool (6 g.) and

ethyl α -bromoacetate (16 g.) was heated on the water-bath for about 2 hours and the solution poured into ice-cold dilute sulphuric acid. The benzene layer was removed, washed with soda solution and then with water, dried, benzene removed and ethyl β -hydroxy- β -(2-methoxy)-phenylpropionate distilled at $150-54^{\circ}/10$ mm. as a colourless thick oil, yield 11 g. The condensation product (11 g.), thus obtained, was converted into the unsaturated ester with thionyl chloride (6 g.) in the presence of pyridine (9 g.) in dry ethereal solution. After decomposing the excess of thionyl chloride with ice the ethereal layer was washed with hydrochloric acid, alkali and then with water. On removing ether ethyl 2-methoxy-*trans*-cinnamate was distilled at $150^{\circ}/8$ mm., yield 10 g. (Found C, 69.8; H, 6.7. $C_{12}H_{14}O_3$ requires C, 69.9; H, 6.79 per cent).

2-Methoxy-*trans*-cinnamic acid was obtained by the hydrolysis of the above ester with alcoholic potash. It was crystallised from alcohol, m.p. 182° . It was identified as 2-methoxy-*trans*-cinnamic acid by preparing an authentic specimen from *o*-coumaric acid, which was methylated by means of dimethyl sulphate and the methyl methoxy-*o*-coumarate hydrolysed by alkali. The two specimens were identical in all respects (m.p. and mixed m.p. 182°).

*Ethyl 2-Methoxy- α -methyl-*trans*-cinnamate*.—Ethyl α -methyl- β -hydroxy- β -(2-methoxy)-phenylpropionate, b.p. $155^{\circ}/4$ mm. was obtained by submitting 2-methoxybenzaldehyde (6 g.) and ethyl α -bromopropionate (6 g.) to Reformatsky's reaction in the presence of zinc wool (3 g.), yield 6 g. It was converted into ethyl 2-methoxy- α -methyl-*trans*-cinnamate with thionyl chloride (8 g.) in the presence of pyridine (5 g.) in the usual manner, b.p. $150-55^{\circ}/4$ mm.

trans-2-Methoxy- α -methylcinnamic acid, m.p. 102° , was obtained by the hydrolysis of the above compound with alcoholic potash (*cf.* Perkin, *loc. cit.*).

*Ethyl 2:4-Dimethoxy-*trans*-cinnamate*.—Ethyl β -hydroxy- β -(2:4-dimethoxy)-phenylpropionate, the condensation product of 2:4-dimethoxybenzaldehyde (6.6 g.) and ethyl α -bromoacetate (8 g.) using zinc wool (4 g.), was obtained as a thick colourless oil, b.p. $180-84^{\circ}/8$ mm., yield 6 g. It was dehydrated with thionyl chloride and pyridine in the usual manner giving the cinnamic ester, b.p. $180-184^{\circ}/8$ mm.

trans-2:4-Dimethoxycinnamic acid, m.p. 184° , was obtained by the hydrolysis of the above ester with alcoholic potash. It is identical with the compound described by Perkin and Schiess (*loc. cit.*).

*Ethyl 2-Methoxy-5-chloro-*trans*-cinnamate*.—Ethyl β -hydroxy- β -(2-methoxy-5-chloro)-phenylpropionate, b.p. $185^{\circ}/4$ mm., was obtained by condensing 2-methoxy-5-chlorobenzaldehyde (8.5 g.), ethyl α -bromoacetate

(8.5 g.) using zinc wool (4 g.) as usual. The propionate (8 g.) was dehydrated with thionyl chloride (4 g.) in the presence of pyridine (6 g.), when the cinnamate was obtained as a colourless oil, b.p. $170^{\circ}/6$ mm. (Found: Cl, 14.3. $C_{12}H_{13}O_3Cl$ requires Cl, 14.76 per cent).

trans-5-Chloro-2-methoxycinnamic acid was obtained by hydrolysing the above ester with potash. It crystallised from alcohol, m.p. 191° . It was identified with a sample of an authentic acid having the *trans*-configuration. 5-Chloro-*o*-coumaric acid, m.p. 190° , was prepared from 6-chloro-coumarin by mercuric oxide according to the method of Sen and Chakravarti (*loc. cit.*). It was then methylated with dimethyl sulphate and alkali and the resulting methyl 5-chloro-2-methoxy-*o*-coumarate hydrolysed with alcoholic potash giving *trans*-5-chloro-2-methoxycinnamic acid, m.p. and mixed m.p. 191° .

Ethyl 2:4-dimethoxy- $\alpha\beta$ -dimethylcinnamate was prepared by condensing resacetophenone dimethyl ether (10 g.), ethyl α -bromopropionate (9 g.) using zinc wool (4 g.). The unsaturated ester was formed by dehydration during vacuum distillation. It boils at $180-82^{\circ}/6$ mm., yield 8.5 g. 3:4-Dimethyl-7-methoxycoumarin was obtained by mixing the above compound with sulphuric acid (*d* 1.84) in the cold and allowing to stand overnight. The solution was poured into powdered ice and the solid separating was collected and crystallised from alcohol as colourless silky needles, m.p. 140° (not depressed when mixed with an authentic specimen). 3:4-Dimethyl-7-hydroxycoumarin was also obtained by heating ethyl 2:4-dimethoxy- $\alpha\beta$ -dimethylcinnamate (1 g.) with hydriodic acid (*d* 1.7, 7 c.c.) at 140° for 2 hours. The mixture was poured into water and the solid obtained was crystallised from alcohol as colourless needles, m.p. 256° , the m.p. being not depressed when mixed with an authentic sample.

Ethyl 2:4-dimethoxy- β -methylcinnamate was prepared by reacting resacetophenone dimethyl ether (10 g.), ethyl α -bromoacetate using zinc wool (4 g.). The hydroxy-ester was dehydrated during vacuum distillation. It was obtained as a light yellow oil, b.p. $174^{\circ}/6$ mm., yield 9 g. 2:4-Dimethoxy- β -methylcinnamic acid, m.p. 145° , was readily obtained by the hydrolysis of the above ester, identical with the acid described by Pechmann and Cohen (*Ber.*, 1884 17, 2132). It has not been possible to effect the ring-closure of the above ester either by heating with hydriodic acid or with cold concentrated sulphuric acid as the oily product obtained could not be crystallised.

ACTION OF OXALYL CHLORIDE ON PHENOLIC ETHERS.

BY P. C. MITTER AND HITENDRANATH MUKHERJEE.

Condensation of oxalyl chloride with phenolic ethers in presence of anhydrous aluminium chloride gives rise to *o*-diketones in the cases of anisole and *o*-cresol-methyl ether, while with *m*-cresol- and *p*-cresol-methyl ethers, only the corresponding acids are obtained. With veratrole protocatechuic acid is obtained

Liebermann and his coworkers have made extensive investigations on the action of oxalyl chloride on aromatic hydrocarbons (*Ber.*, 1911, **44**, 202, 852, 1453; 1912, **45**, 1186; 1913, **46**, 198) in presence of anhydrous aluminium chloride and obtained in a majority of cases the corresponding monocarboxylic acids. In a few cases, for instance with anthracene and *pp*-ditolyl, 1:2-diketones were obtained. We have investigated the action of oxalyl chloride on phenolic ethers, *e.g.*, anisole, *o*-, *m*- and *p*-cresol-methyl ethers and veratrole. With anisole, the diketone (anisil) is obtained in about 90% yield. This, by the way, seems to be the best method of preparing the substance. The diketone is identified by taking a mixed m. p. with a sample at hand and also by oxidising it with hydrogen peroxide to anisic acid (Holleman, *Chem. Zentr.*, 1904, **II**, 194).

With *o*-cresol methyl ether also a diketone is obtained along with a trace of an acid which could not be identified. The diketone gives on oxidation with hydrogen peroxide 4-methoxy-*m*-toluic acid, m. p. 192-93°. Its methyl ester melts at 67°

m-Cresol methyl ether gives under exactly similar conditions 6-methylsalicylic acid, m. p. 167°. With *p*-cresol methyl ether also no diketone is obtained and 5-methylsalicylic acid, m. p. 149°, is the only product of the reaction. Veratrole gives protocatechuic acid, m. p. 189°, and no diketone is obtained.

Resorcinol dimethyl ether gives a tarry matter from which no product could be isolated. Hydroquinone dimethyl ether does not react at all, while with pyrogallol trimethyl ether, an acid mixture is obtained melting through a wide range of temperature, which could not be characterised.

EXPERIMENTAL.

Condensation of Oxalyl Chloride with Anisole: Formation of Anisil.—In a three-necked flask fitted with a reflux condenser provided with a calcium chloride guard tube and a mechanical stirrer with mercury seal,

anisole (10 g.) was dissolved in carbon bisulphide (50 c. c.) and oxalyl chloride (6.3 g.) was added. The solution was cooled by means of a freezing mixture and freshly prepared and finely powdered aluminium chloride (20 g.) was added a little at a time, with vigorous stirring. Hydrochloric acid gas was evolved and after sometime a sticky solid separated at the bottom of the flask. The mixture was allowed to stand overnight, carbon bisulphide was removed by decantation and the reddish brown solid was treated with ice and hydrochloric acid and then heated on the water-bath. The solid was filtered, washed with water, digested with sodium carbonate solution and finally washed with water and crystallised from dilute alcohol as greenish yellow needles, m. p. 132° (mixed m. p. with anisil), yield 90%. (Found: C, 71.06; H, 5.18. Calc. for $C_{18}H_{14}O_4$: C, 71.12; H, 5.17 per cent).

The sodium carbonate washings on acidification with dilute hydrochloric acid and extraction with ether gave a small quantity of a solid, m. p. 153° (mixed m. p. with salicylic acid).

Oxidation of Anisil.—A solution of anisil (3 g.) in glacial acetic acid was treated with perhydrol (15 c. c.) and the whole heated at $70-80^{\circ}$ for about 4 hours. The solution on cooling deposited white needles of anisic acid and a further quantity was obtained by diluting the filtrate. The combined solids were washed, dissolved in soda solution, reprecipitated with dilute hydrochloric acid and the acid recrystallised from hot water (charcoal), m. p. 183° , yield about 2 g. (Found: C, 62.8; H, 5.37. Calc. for $C_8H_8O_3$: C, 63.17; H, 5.26 per cent).

Condensation of Oxalyl Chloride with o-Cresol methyl ether.—A mixture of *o*-cresol methyl ether (10 g.) in carbon bisulphide (50 c. c.) and oxalyl chloride (5 c. c.) was treated with anhydrous aluminium chloride (20 g.) and the product treated as in the previous case, m. p. 174° , yield of diketone about 5 g. (Found: C, 72.57; H, 5.33. $C_{18}H_{18}O_4$ requires C, 72.48; H, 5.04 per cent).

Oxidation of the diketone (2 g.) with hydrogen peroxide gave 1.5 g. of 4-methoxy-*m* toluic acid. (Found: C, 65.43; H, 6.06. Calc. for $C_9H_{10}O_3$: C, 65.06; H, 6.02 per cent).

4:4'-Dimethoxy-3:3'-dimethylbenzilic Acid.—The above diketone (3 g.) was added to caustic soda (10 g.) and heated to about 180° in a nickel basin. It melted and the mixture solidified to a reddish substance. The cooled melt was dissolved in water and acidified with hydrochloric acid. The precipitated acid was filtered, washed with water and recrystallised from hot water (charcoal), m. p. $145-47^{\circ}$. (Found: C, 68.52; H, 6.84. $C_{18}H_{20}O_5$ requires C, 68.37; H, 6.33 per cent).

Condensation of Oxalyl Chloride with p-Cresol methyl ether.—The condensation was effected as in the previous cases but no diketone was obtained. With *p*-cresol methyl ether (10 g.), oxalyl chloride (10 c.c.) and aluminium chloride (15 g.) 5-methylsalicylic acid (1.6 g.), m.p. 149°, was the only product of the reaction. (Found: C, 64.52; H, 5.23. Calc. for $C_8H_8O_3$: C, 63.17; H, 5.26 per cent).

Condensation of Oxalyl Chloride with m-Cresol methyl ether.—A mixture of *m*-cresol methyl ether (10 c.c.), oxalyl chloride (10 c.c.) and carbon bisulphide (100 c.c.) was treated with aluminium chloride (20 g.) in the usual way. The only product was 3-methylsalicylic acid, m.p. 167-68°. (Found: C, 62.69; H, 5.44. Calc. for $C_8H_8O_3$: C, 63.17; H, 5.26 per cent).

Condensation of Oxalyl Chloride with Veratrole.—A mixture of veratrole (10 c.c.), oxalyl chloride (10 c.c.) and carbon bisulphide (75 c.c.) was treated with aluminium chloride (15 g.) in the usual way. The only product was protocatechuic acid, m.p. 189°, yield 2 g.

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Received June 21, 1939.

STRYCHNINE AND BRUCINE. PART I. THE ALKALINE DEGRADATION OF STRYCHNINE.

By RAFAT HUSAIN SIDDIQUI.

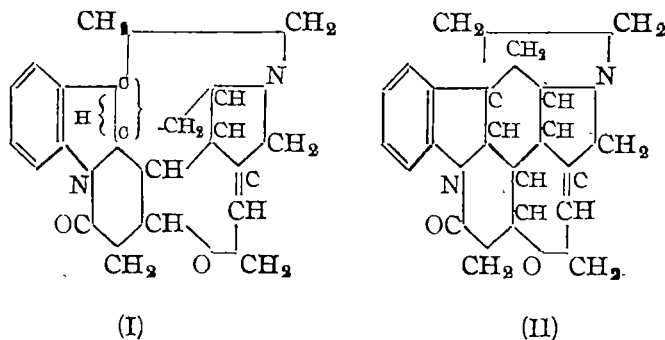
Distillation of strychnine with potassium hydroxide gives among the products a base (picrate, m.p. 142°), which is identical with the base A described by Clemo.

Goldschmidt (*Ber.*, 1882, **15**, 1977) obtained indole and an oil which he called γ -picoline by fusing strychnine with caustic potash. Stöhr (*Ber.*, 1887, **20**, 810, 1808) obtained scatole, β -picoline, and ethylpyridine as degradation products but Mofatti and Löbisch obtained β -picoline, carbazole and scatole (*Monatsh.*, 1888, **9**, 626) under similar conditions. Clemo, Perkin and Robinson (*J. Chem. Soc.*, 1927, 1589) isolated indole, carbazole and a picrate of a base which melted at 168° . Kotake (*Proc. Acad. Tokyo*, 1936, **12**, 4, 99) announced the important discovery of the formation of 3-indolyethylamine by fusion of strychninolone, strychninonic acid or even strychnine with potassium hydroxide. After this announcement Clemo (*J. Chem. Soc.*, 1936, 1695) reported the isolation of basic and non-basic products by fusing strychnine with potassium hydroxide under very mild conditions. Among the non-basic products, he identified indole and 3-ethylindole. Two of the three basic products which he isolated remained unidentified and the third in all probability seemed to be tryptamine, but on account of the conflicting data in the literature, the final recognition of the substance was temporarily held up.

The present work was started in November, 1935 but owing to the publication of the above papers it was discontinued. However, along with other products one base was isolated which seemed to be common both to strychnine and brucine and different from that obtained by Kotake. This was purified through its picrate and subsequently by fractional distillation; the picrate (m.p. $141-42^{\circ}$) appeared from its melting point and analytical data to be the picrate of 2-methyl-4-ethylpyridine. 2-Methyl-4-ethylpyridine was synthesised by Hantzsch's method but its picrate (m.p. 142°) depressed the m.p. of the picrate of the strychnine degradation base and melted at $115-125^{\circ}$. Thus it showed conclusively that it was not 2-methyl-4-ethylpyridine but some other isomer of it. Clemo reports that the m.p. and the mixed m.p. of the picrate of his base and the foregoing picrate to be $143-44^{\circ}$ (*private communication*), thus establishing the identity of the two.

The author obtained the base under drastic conditions by distilling the intimate mixture of strychnine and potassium hydroxide in a copper flask,

while Clemo carried out his experiment under very mild conditions. Credit must be given to Clemo and Kotake, who independently isolated β -3-indolyl-ethylamine and this observation justifies the consistent contention of Robinson that strychnine and brucine are hydroindole derivatives in which the positions 2 and 3 (α - and β -) are thrice substituted (*cf. J. Chem. Soc.*, 1937, 941) and carry but a single hydrogen atom. This observation was interpreted by a slightly modified formula (I) for strychnine by the author as suggested by Robinson (*cf. Oxford University Thesis for D.Phil.*, 1937) but Holmes and Robinson's re-examination of the action of bromine on diketonucidine is an important contribution on the subject and now the balance of evidence is in favour of formula (II) (*cf. J. Chem. Soc.*, 1939, 603).



EXPERIMENTAL

Fusion of Strychnine with Caustic Potash (*cf. Abstracts of Dissertations*, Oxford, 1938, 173).

Strychnine (1 part) was intimately mixed with powdered potassium hydroxide (3 parts) and water (1 part) and the paste distilled from a copper flask heated by a gradually strengthened free flame. Water and a straw coloured oil with a strong odour distilled over. The distillate was extracted with ether and the ethereal solution was shaken with 10% hydrochloric acid. The ether extract, after washing with sodium hydroxide solution and water, was dried over anhydrous sodium sulphate and on removal of solvent gave an oily neutral product with a strong odour. This was not investigated further.

The acid extract was made alkaline with sodium hydroxide and was shaken with ether four times. The ethereal solution was washed with water and dried over anhydrous sodium sulphate. The addition of picric acid in ether to the dried ethereal solution precipitated a bright yellow picrate which was well washed with ether. The filtrate and washings on standing

gave another bright yellow picrate, m.p. 186-87°. After two crystallisations from methyl alcohol it had m.p. 195-96°. (Found in material dried at 100° in *vacuo*: C, 63.6; H, 4.1; N, 16.8 per cent). The picrate was insufficient for further characterisation.

The main bright yellow picrate was decomposed with sodium hydroxide solution and water and then shaken four times with ether. The ethereal extract was washed with water and dried over solid potassium hydroxide. On removal of the solvent, an oily straw-coloured liquid remained which was fractionally distilled. The lower boiling fraction was converted into its picrate and after crystallisation from alcohol several times was obtained in large bright yellow leaflets, m. p. 141-42°. (Clemo found the m.p. and the mixed m.p. with the picrate of his base "A" to be 143-44°). It is soluble in alcohol, acetone, chloroform, benzene and ethyl acetate, and insoluble in ether and petroleum ether. [Found in material dried at 100° in *vacuo*: C, 48.0, 47.9; H, 4.3, 4.0; N, 15.2, 15.3. $C_8H_{11}N \cdot C_6H_2(NO_2)_3(OH)$ requires C, 48.0; H, 4.0; N, 16.0 per cent].

The micro-analyses were done by Dr. Weiller and Dr. Strauss, Oxford.

The author is grateful to Professor Sir Robert Robinson, Kt., F.R.S. for his advice and encouragement throughout this investigation, to Professor G. R. Clemo, F.R.S. for the comparison of the melting points and to the Trustees, Dawoodbhoy Fazalbhoy Muslim Educational Trust, Bombay, for an Educational loan which enabled the author to take part in this investigation.

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Received July 14, 1939.

STRYCHNINE AND BRUCINE. PART II THE ALKALINE DEGRADATION OF BRUCINE.

BY RAFAT HUSAIN SIDDIQUI.

The basic fraction obtained by the alkaline degradation of brucine has been fractionated and picrates have been obtained from these fractions. Among the products a base (picrate, m. p. 142°) is common to strychnine and brucine and is also identical with the base A described by Clemo.

Oechner (*Ann. chim. phys.* 1882, v, 27, 507) investigated the action of potassium hydroxide on brucine (for details vide *Compt. rend.*, 1882, 99, 1077; *Bull. Soc. chim.*, 1881, 42, 100). Later Brend and Stöhr (*J. pr., Chem.*, 1890, ii, 42, 4161) by distilling brucine with soda lime obtained ammonia, methylamine, β -picoline, and β -ethylpyridine which they analysed as mercury salt. In the present work brucine has been distilled with potassium hydroxide and the basic fraction, isolated from the distillate, has been distilled in *vacuo* and then fractionated into six fractions of which the last is too small for investigation. The remaining five fractions have been converted into picrates.

Fraction I gives a picrate which after repeated crystallisation is obtained in shining bright yellow prisms, m. p. $143-44^{\circ}$ (m. p. $145-48^{\circ}$ as found by Clemo, *private communication*). It gives analytical values in agreement with C_7H_9N but the m. p. of the picrate does not correspond to the picrate of any of the lutidines. The b. p. is close to that of 2-ethyl-lutidine but the m. p. of the picrate of this base is not recorded in the literature.

Fraction II gives a mixture of picrates, m. p. *circa* $104-5^{\circ}$.

Fraction III gives a picrate which melts at $141-42^{\circ}$ (m. p. $143-44^{\circ}$ found by Clemo) and does not depress the m. p. of the picrate obtained from strychnine by fusion with potassium hydroxide (*vide* previous part). The m. p. of the picrate and the boiling point of the base corresponds to that of the picrate of 2-methyl-4-ethylpyridine and the free base but differs when directly compared (*vide* Part I).

Fraction IV mostly gives the picrate, m. p. $141-42^{\circ}$ and gives no depression on admixture with the picrate of fraction III.

Fraction V distills at $50^{\circ}/1.5$ mm. and the picrate obtained from it melts at 172° . Its analysis agrees with $C_{11}H_{11}N$ or $C_{11}H_{13}N$.

The filtrates from different fractions yield crystalline bright yellow picrates of varying melting points. Judging from their general behaviour,

solubilities and smell, it can safely be assumed that they are pyridine derivatives.

In the present state of our knowledge brucine can be represented as dimethoxy derivative of strychnine having the formula (II) of the previous paper.

EXPERIMENTAL.

Fusion of Brucine with Caustic Potash (cf. *Abstracts of Dissertations*, Oxford, 1938, p. 173).—

Brucine (1 part), mixed with powdered potassium hydroxide (2 parts), was placed in a copper flask over a layer of potassium hydroxide (1 part), and the top covered with further amount (1 part) of the alkali. The flask was fitted with a condenser and a receiver cooled in ice. An oil with some water distilled over on gradual heating and the evolved gases were found to be strongly alkaline to litmus. The distillate was shaken with ether and the ethereal solution extracted with 10% dilute hydrochloric acid. The acid extract was made alkaline with sodium hydroxide and extracted with ether. The ethereal solution after washing with water and drying over solid potassium hydroxide furnished a brownish yellow oil as residue, yield 5%. On distillation in *vacuo* it was obtained as a light pale yellow liquid with a penetrating odour which deepened in colour on standing. It was next distilled at atmospheric pressure and collected in six fractions of which the last was very small in amount.

Fraction I (b. p. 145-50°/764 mm., 5 g.), in ether (50 c.c.) was precipitated with ethereal picric acid solution, and the bright yellow picrate separated in prisms and plates. It was well washed with ether (8 g.), m. p. 124-25°. A further quantity (3.5 g., m. p. 124-25°) was obtained from the filtrate. The combined fractions were crystallised from methyl alcohol several times. It was finally obtained in glistening bright yellow prisms, m. p. 143-44° (m. p. 145-48° as found by Clemo). It is soluble in acetone, alcohol, warm water, difficultly in ethyl acetate, benzene and chloroform and is insoluble in petroleum ether. After drying at 100° in *vacuo* it suffered no loss in weight. [Found: C, 45.9, 45.8; H, 3.5, 3.1; N, 16.7, 17.0. $C_7H_6N \cdot C_6H_2(NO_2)_3(OH)$ requires C, 46.4; H, 3.6; N, 16.7 per cent].

Fraction II (b. p. 155-65°/764 mm., 0.65 g.) was similarly converted into a bright yellow picrate which on recrystallisation from methyl alcohol melted at 104.5° with gradual softening. It appears to be a mixture of fraction I and fraction III.

Fraction III (b. p. 170-175°/764 mm., 2.7 g.) gave a bright yellow picrate (4.9 g.), m. p. *circa* 104° and the orange filtrate and washings showed

a green fluorescence. On crystallising several times from methyl alcohol it melted at $135-36^{\circ}$. It gives no appreciable depression with the picrate of the fraction IV (m.p. $141-42^{\circ}$) but when mixed with the picrate of fraction I it melted at $105-12^{\circ}$.

Fraction IV (b.p. $180^{\circ}/764$ mm., 3.8 g.) was dissolved in ether and on adding ethereal solution of picric acid bright yellow prisms were obtained after washing with ether (5 g.). The picrate melted at $120-24^{\circ}$ but on crystallising four times from methyl alcohol it was obtained in long shining lemon-yellow plates and leaflets, m. p. $141-42^{\circ}$ and three subsequent crystallisations did not raise the m. p. further. It showed depression when admixed with the picrate of the above obtained from strychnine (*vide* Part I).

Fraction V (b. p. $50-53^{\circ}/1.5$ mm.) showed a violet fluorescence alone or in ether solution. The picrate isolated from ether solution in the usual manner was obtained as a sticky precipitate which dissolved in alcohol leaving some dark impurities. From the orange-red solution the picrate crystallised on keeping, m. p. $128-30^{\circ}$. After repeated crystallisations from methyl alcohol it was obtained as woolly yellow prisms, m. p. 172° after softening at $163-168^{\circ}$. After drying at 100° in *vacuo* it lost 2.6% (loss of $\frac{1}{2}$ H_2O requires 2.3 per cent). It depressed the m. p. of the picrates of quinoline and isoquinoline and differed from them in other details also. [Found in anhydrous material: C, 52.3, 52.4; H, 3.9, 4.0; N, 13.8, 14.0. $C_{11}H_{11}N \cdot C_6H_2(NO_2)_3(OH)$ requires C, 52.8; H, 3.6; N, 14.5 per cent. $C_{11}H_{13}N \cdot C_6H_2(NO_2)_3(OH)$ requires C, 52.6; H, 4.1; N, 14.4 per cent].

The filtrates from all the above fractions yielded crystalline bright yellow picrates of varying melting points and were not investigated.

The micro-analyses were done by Dr. Weiller and Dr. Strauss, Oxford.

The author is grateful to Professor Sir Robert Robinson, Kt, F.R.S. for his advice and encouragement throughout this investigation, to Professor G. R. Clemo for the comparison of the m.p. and to the 'Trustees, Dawoodbhoy Fazalbhoy Muslim Educational Trust, Bombay, for an educational loan which enabled the author to take part in this investigation.

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Received July 14, 1939

STUDIES IN LONG-CHAIN ACIDS. PART I. AN EXTENSION OF THE ISOPRENE RULE.

By P. C. MITTER AND PHANINDRA NATH BAGCHI.

The isoprene rule has been extended so as to explain the formation of some 12- and 16-carbon acids occurring in nature.

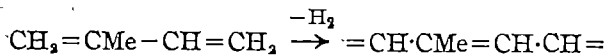
Isoprene has been long recognised as one of the chief building units utilised by nature in the synthesis of plant products and also to a lesser extent in the synthesis of animal products.

The formation of larger molecules from isoprene is assumed to occur in one of the three following ways :—

(1) By direct linear head to tail union, a polymerisation which may result in either acyclic or cyclic hydrocarbons and which is generally recognised as the origin of the terpenes $(C_5H_8)_n$.

(2) By a similar type of polymerisation with concurrent hydrogenation which would explain the formation of such hydrocarbons as that from which the alcohol, phytol $(C_{20}H_{40}O)$ is derived.

(3) By addition with accompanying dehydrogenation in 1:4 position,

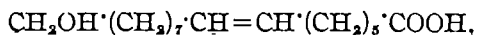


These C_5H_8 units then combine to longer chains as in the carotenoids. (Kuhn and Winterstein, *Helv. Chim. Acta*, 1928, 11, 430).

The formation of a large number of long-chain aliphatic acids, both mono- and dibasic can be explained if to the above three we add a fourth category namely :—

(4) Head to tail union of isoprene units ; addition of H_2O at a conjugated double bond at one end of the chain ; partial or complete hydrogenation and removal of side-chain methyl groups by oxidation and oxidation, complete or partial, of the terminal groups.

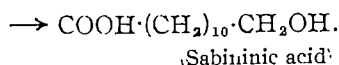
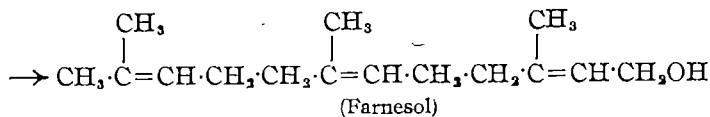
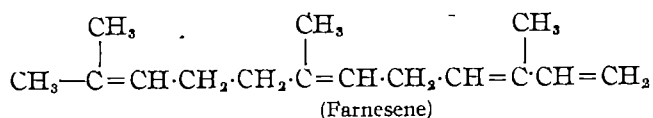
The facts which led to this hypothesis are briefly these : Kerschbaum (*Ber.*, 1927, 60, 902), had detected the presence of farnesol along with ambrettolid in musk kernel oil and in connection with our work on aleuritic and ambrettolic acids (to be published later) we were anxious to connect the presence of farnesol with that of the lactone ambrettolid. Now, the constitution of ambrettolic acid (*loc. cit.*) has been found to be



It is a straight chain 16-carbon acid while its dihydro derivative (juniperic acid) has been found to be associated in nature with sabbinic acid, a straight chain 12-carbon acid, in the wax of *Juniperus Sabina* by Bougault and Bourdier (*Chem. Zentr.*, 1909, **II**, 718).

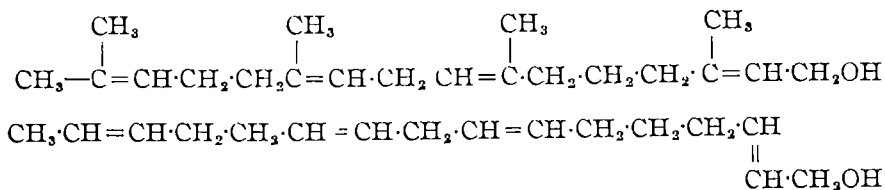
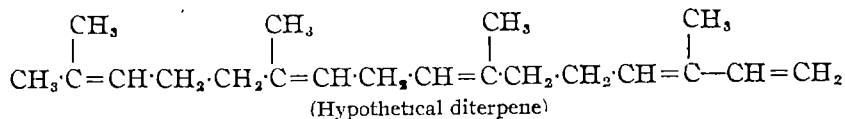
Evidently the common building unit in these two acids contains a chain of four carbon atoms and if, as is likely to be the case, isoprene is the building unit, a side-chain methyl group per unit must have been removed by oxidation.

Inspection of the formula of farnesol, which, by the way, may be regarded as derived from farnesene by the addition of a molecule of water at a conjugated double bond, reveals its close connection with sabinic acid.



As a matter of fact, removal of the side-chain methyl groups by oxidation, hydrogenation of the double bonds and oxidation of a terminal methyl group leads from farnesol to sabinic acid.

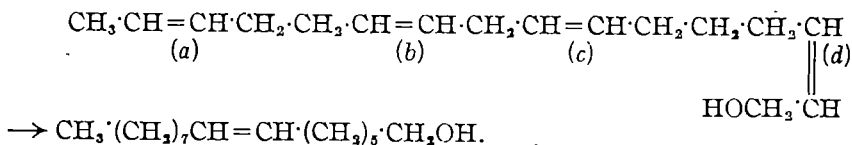
Viewed from this stand-point, juniperic acid would be derivable from a diterpene $C_{30}H_{52}$, with a constitution similar to that of farnesene, by a similar mechanism.



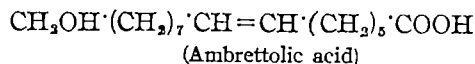
Reduction of all the double bonds and oxidation of the methyl group leads to $\text{COOH}(\text{CH}_2)_{14}\text{CH}_2\text{OH}$ (juniperic acid) while oxidation of both the terminal

groups leads to $\text{COOH} \cdot (\text{CH}_2)_{14} \cdot \text{COOH}$ (thapsic acid) obtained by Canzoneri from *Thapsia* resin (*Gazzetta*, 1883, 13, 514; cf. *Chem Zentr.*, 1920, II, 710; 1923, I, 502).

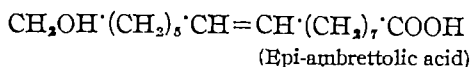
A very interesting point which lends additional support to the hypothesis is brought out if we consider the effect of incomplete hydrogenation namely that of the double bonds marked (a), (b) and (d)



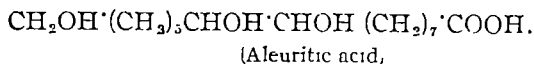
Here the oxidation of the terminal methyl group to CH_2OH and of the terminal CH_2OH group to COOH leads to



while the oxidation of the terminal methyl group alone to COOH leads to an epimer of ambrettolic acid (paper to follow)



very closely related to another important acid present in shellac resin



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Received August 5, 1939.

ROTTLERIN. PART IV. DERIVATIVES OF ISOROTTLERIN.

BY RAVI SARUP JALOTA, KARTAR SINGH NARANG AND JNANENDRA NATH RÂY.

*iso*Rottlerin, described by Brockmann and Maier, is identical with the colouring matter (m. p. 181°) of Narang, Ray and Roy. Separation of rottlerin from *isorottlerin* is best effected chromatographically. Rottlerin can also be converted into *isorottlerin* by means of hydrochloric acid. *iso*Rottlerin gives a piperonylidene derivative indicating the presence of a $-\text{COCH}_3$ group.

In Part I (*J. Chem. Soc.*, 1937, 1862) Narang, Ray and Roy obtained a substance, m. p. 208-9° (decomp.) by the action of nitrous acid on rottlerin methyl ether. They suggested that the substance could be represented by the formula $\text{C}_{17}\text{H}_{19}\text{O}_5\text{N}$ or $\text{C}_{19}\text{H}_{21}\text{O}_6\text{N}$. Later on, they obtained 15.5 g. of this substance from 17 g. of rottlerin methyl ether. This indicated that the substance in question is a simple N_2O_3 -addition product and the molecule has not undergone any breakdown. Further the N_2O_3 -adduct (nitrosite) gave a dihydro derivative (dihydronitrosite) on catalytic reduction. The nitrosite and dihydronitrosite on treatment with alkali gave isomers, m. p. 152-53° and 139° respectively.

From a study of the nitrosite and its derivatives as also from the analytical data of oxide of rottlerin methyl ether, Narang, Ray and Roy came to the conclusion that rottlerin is best represented by the formula $\text{C}_{31}\text{H}_{30}\text{O}_8$ with five hydroxyl groups. They have expressed this view in their various publications (*Chem. Ind.*, 1938, 57, 134; *Current Science*, 1938, 6, 333; *J. Indian Chem. Soc.*, 1938, 15, 393).

Narang, Ray and Roy mentioned the presence of a second yellow colouring matter, (m. p. 181°) in Kamala powder (*J. Chem. Soc.*, 1937, 1863). This substance has now been more fully investigated. We have found that it can be easily separated from rottlerin if the crude rottlerin is crystallised from toluene. The toluene filtrate on concentration deposits a sticky mass which on dissolution in ether and chromatographic adsorption on alumina, separates into six zones. The first dark zone is that of rottlerin, while the second zone contains the substance, m. p. 181°. We have obtained this very substance by treating rottlerin with aqueous alcoholic hydrochloric acid. We have now come to the conclusion that this substance is identical with the substance, m. p. 180°, described by Brockmann and Maier (*Annalen*, 1938, 535, 170) subsequent to our isolation of the substance in 1937.

Brockmann and Maier (who have named this substance *isorottlerin*) have described it as a flavanone formed by isomerisation of a hydroxy-chalcone group in the molecule. At first we were also inclined to this view because *isorottlerin* methyl ether, m.p. 135-37°, gives a piperonylidene derivative and also on reduction it takes up only two atoms of hydrogen giving mainly a dihydro derivative, m.p. 209°. Therefore, it seems that one of the double bonds is involved in the formation of *isorottlerin* from *rottlerin* and a COCH_2 group is formed in the molecule which react to give the piperonylidene derivative. But the oxide of *isorottlerin* methyl ether, m.p. 120-22°, on being heated above its m.p. gives off benzaldehyde copiously. Therefore, it seems unlikely that $-\text{COCH}=\text{CHPh}$ is involved in isomerisation. It might be mentioned here that nitrosite of *isorottlerin* methyl ether is recovered unchanged when subjected to catalytic reduction and it also gives off benzaldehyde on heating, or on treatment with alkali.

We have reduced *isorottlerin* methyl ether with zinc dust and acetic acid and isolated a substance, m.p. 162-64°. Dihydro*isorottlerin* methyl ether on similar treatment gives the same substance. This suggests that besides the double bond in *isorottlerin* methyl ether, some other group is involved in this reduction. We are engaged in elucidating this point.

For the sake of comparison we have also reduced *rottlerin* methyl ether with zinc dust and acetic acid and obtained a product, m.p. 184° (previous shrinking from 179°). It could not be acetylated with acetic anhydride and pyridine or oxidised with hydrogen peroxide and alkali. This shows that a $-\text{CHOH}$ group has not been created in the process of reduction and the double bond in the $-\text{COCH}=\text{CHPh}$ group has been affected. We are investigating the nature of these compounds. Oximation of *isorottlerin* methyl ether did not prove successful. The ethereal or acetic acid solution of methyl ether of *isorottlerin* on treatment with salicylaldehyde in presence of hydrogen chloride gives a permanganate-violet coloured solution but nothing crystalline separates. We are unable to say if a pyryllium salt is definitely formed.

EXPERIMENTAL.

isoRottlerin.—(a) The mother-liquor left after crystallisation of *rottlerin* from toluene was concentrated. 15 G. of the residue were treated with 150 c.c. of ether. The insoluble *isorottlerin* was collected, washed with petroleum ether and crystallised from a mixture of methyl alcohol and ethyl acetate, m.p. 180-81°.

(b) 85 G. of the residue mentioned in the above experiment were dissolved in 250 c.c. of ether. This was run through a layer of alumina set

in a long tube of 2 cm. diameter. Six zones were formed and a waxy substance was isolated from the colourless ethereal solution. The first dark zone was that of rottlerin; the second zone on elution with a mixture of ether and alcohol gave *isorottlerin*, which crystallised in pale yellow prisms, m.p. and mixed m.p. with substance isolated in (a), 180-81°. (Found: C, 69.95; H, 5.57. $C_{31}H_{30}O_8$ requires C, 70.19; H, 5.66 per cent).

Conversion of Rottlerin into isoRottlerin.—A mixture of rottlerin (5 g.), alcohol (250 c.c. of 90%) and hydrochloric acid (15 c.c., d 1.15) was heated for 7 hours, the solution becoming clear after 3½ hours. The mixture on standing overnight deposited a dark red solid. The filtrate on dilution with water gave a pale yellow solid which was collected, dissolved in ether and the ethereal solution washed with sodium bicarbonate. After drying, the ethereal solution was concentrated, when the yellow solid crystallised out. This was found to be identical with the substance, m.p. 181° (mixed m.p.) obtained from natural resources. In later experiments, the yellow solid, as obtained by dilution, was purified by chromatographic absorption, yield 2g. *isoRottlerin* is very soluble in acetone, ethyl methyl ketone and chloroform. The substance as obtained in this experiment is best crystallised from a mixture of 90% alcohol and 10% benzene.

Methylation of Natural isoRottlerin.—A mixture of the natural *isorottlerin* (isolated as under a or b) (1 g.), dry potassium bicarbonate (8 g.), dimethyl sulphate (4 c.c.) and acetone (50 c.c.) was refluxed on a steam-bath for 2½ hours when the colour of the solution became pale. Dry potassium carbonate (4 g.) and 2 c.c. of dimethyl sulphate were now introduced and the heating continued for another 1½ hours when the colour of the solution became faintly yellow. Acetone was distilled off and the residue left in contact with water overnight. The semi-solid mass was collected and successively crystallised from petroleum ether and methyl alcohol, m.p. 135-38° and not 105° as wrongly reported previously (*vide*, J. Chem. Soc., 1937, 1863). (Found: C, 71.64; H, 6.42. *isoRottlerin* tetramethyl ether, $C_{35}H_{38}O_8$ requires C, 71.67; H, 6.42% and *isorottlerin* pentamethyl ether, $C_{36}H_{40}O_8$ requires C, 72.0; H, 6.66 per cent).

Methylation of isoRottlerin Prepared from Rottlerin.—The methylation and purification of the product was carried out exactly in the same manner as in the case of the natural *isorottlerin*, m.p. 135-38°, undepressed by admixture with methyl ether obtained from the natural product. (Found: C, 71.54; H, 6.73 per cent).

A solution of piperonal (0.15 g.) and *isorottlerin* methyl ether (0.6 g.) in alcohol (8 c.c.) was boiled for 2 minutes under reflux with sodium hydroxide solution (10%, 1 mol). After the removal of most of the solvent, the

product was washed with water and crystallised in rectangular plates, m.p. 147° from ethanol. (Found : C, 71.9; H, 5.9. $C_{43}H_{42}O_{10}$ requires C, 71.8; H, 5.8% and $C_{44}H_{44}O_{10}$ requires C, 72.1; H, 6.0 per cent).

Oxidation of isoRottlerin methyl ether with Alkaline Hydrogen Peroxide.

—The methyl ether (0.5 g.) dissolved in methyl alcohol (90 c.c.) was treated with 3 c.c. of 8% sodium hydroxide solution and 4 c.c. of hydrogen peroxide (30%). The solution was warmed to 50° for $\frac{1}{2}$ hour and then left overnight. The colourless solid which separated was collected and successively crystallised from dilute and absolute methyl alcohol. It was obtained in long colourless prisms, m.p. $120-22^{\circ}$. The oxide, when heated above its m.p., gives off copious amounts of benzaldehyde. (Found : C, 69.99, 69.90; H, 6.43, 6.71. *isoRottlerin* tetramethyl ether oxide, $C_{35}H_{38}O_8$, requires C, 69.77; H, 6.33 per cent., whereas oxide of pentamethyl ether, $C_{38}H_{40}O_8$, requires C, 70.01; H, 6.5 per cent).

Admixed with the oxide of rottlerin methyl ether, m.p. $127-29^{\circ}$ (*J. Chem. Soc.*, 1937, 1864) it melted indefinitely at $105-110^{\circ}$.

Catalytic Reduction of isoRottlerin.—2 G. of *isoRottlerin*, m.p. 181° (purified by repeated crystallisation) were reduced with Adam's platonic oxide catalyst in ethyl acetate solution, when 70 c.c. of hydrogen were absorbed at N.T.P. (one double bond requires 90 c.c. of hydrogen). The crude substance, obtained on removal of the solvent, was crystallised from acetic acid in colourless cubes, m.p. 209° . Reduction with palladium-charcoal also gave the same substance. If, however, only *once purified isorottlerin* (1 g.), m.p. $179-80^{\circ}$, was used, then about 130 c.c. of hydrogen were absorbed and on concentration of the solvent (ethyl acetate) a pale yellow substance (0.1 g.) separated. The mother-liquor on evaporation yielded the dihydroisorottlerin, m.p. 209° (0.7 g.). The pale yellow substance crystallised from ethyl acetate in star-shaped bunches of needles, m.p. $225-28^{\circ}$. [Found in substance (m.p. 208°): C, 69.72; H, 6.02. $C_{31}H_{32}O_8$ requires C, 69.92; H, 6.01 per cent]. This experiment was repeated several times and in every case the product, m.p. $225-28^{\circ}$, was isolated. The product (m.p. $225-28^{\circ}$) gave [C, 68.91, H, 6.41. $(C_{11}H_{12}O_3)_n$ requires C, 68.7; H, 6.21 per cent. $C_{22}H_{26}O_8$ requires C, 69.1; H, 5.8%. $C_{22}H_{26}O_6$ requires C, 68.4; H, 6.7 per cent]. The *isorottlerin* used in this experiment did not appear to have more than traces of impurity as a chromatogram did not reveal any detectable second zone. Therefore, for the time being we assume that traces of impurity in the *isorottlerin* used have acted as a promoter of hydrogenolysis of the molecule and the substance is probably $C_{22}H_{24}O_6$. The C_{11} -formula seems improbable on various grounds. The substance and its mode of formation are being thoroughly examined. If it proves to be C_{22} then

our view of the C_{31} -formula for rottlerin would receive support from the consideration that $C_{22} + C_9$ (i.e. $C_6H_5CH=CH\cdot C$: extruded) makes C_{31} .

Nitrosite of isoRottlerin methyl ether.—Sodium nitrite (0.5 g.) was gradually added during 10 minutes to a solution of 0.5 g. of the methyl ether in 7.5 c.c. of acetic acid at 30° . After standing overnight the reaction mixture was diluted with water to 150 c.c., when a pale yellow substance was obtained which on crystallisation from a mixture of benzene and petroleum ether and finally from absolute alcohol furnished pale yellow prisms, m.p. $194-97^\circ$ (decomp.). [Found: C, 63.36; H, 6.03. $C_{35}H_{38}O_{11}N_2$ (tetramethyl ether) requires C, 63.44; H, 5.74 per cent., while $C_{36}H_{40}O_{11}N_2$ (pentamethyl ether) requires C, 63.92; H, 5.92 per cent.]

Reduction of isoRottlerin methyl ether with Zinc dust and Acetic Acid.—Zinc dust (5 g.) was added during $\frac{1}{2}$ hour to a gently boiling solution of 2 g. of the methyl ether in 40 c.c. of acetic acid. The filtrate on dilution to 250 c.c. with water furnished a white solid which crystallised from a mixture of alcohol and benzene in plates, m.p. $162-64^\circ$ (reserved for further investigation). Dihydroisorottlerin methyl ether, m.p. 209° , on similar treatment gave the same product. The substance gave a dichromate yellow colour with sulphuric acid which became dark red on warming.

For the sake of comparison, rottlerin methyl ether was reduced in a similar manner. The substance crystallised from a mixture of alcohol and ethyl acetate in irregular plates, m.p. 184° (softening from 179°). Repeated crystallisations did not improve the m.p. (Found: C, 71.9, 71.96; H, 6.78, 6.85. $C_{36}H_{42}O_8$ requires C, 71.6; H, 6.97 per cent). We will comment on this result later on. An attempt was made for catalytic reduction of the substance, m.p. 184° , with Adam's catalyst, but the original substance was recovered unchanged.

We also attempted to acetylate the substance, m.p. $178-84^\circ$, but it was unaffected. It was also not affected by alkaline hydrogen peroxide, showing that the $C_6H_5\cdot CH=CH\cdot CO$ -group is no longer present in the molecule. The non-formation of an acetyl derivative indicates that no alcoholic group is present in the molecule. Further work is in progress but the results recorded in this paper clearly demonstrate the invalidity of the rottlerin formulæ proposed by Brockmann and Maier (*loc. cit.*) and by Robertson (*J. Chem. Soc.*, 1938, 309)

STUDIES IN THE PYRIDINE SERIES. PART I. AN ATTEMPT TO SYNTHESISE 2-METHYL-4-ETHYLPYRIDINE.

BY RAFAT HUSAIN SIDDIQUI.

2 : 6-Dimethyl-4-ethyl-1 : 4 dihydropyridine-3 : 5-dicarboxylate has been obtained by condensing ethyl acetoacetate, propionaldehyde, ammonia in presence of piperidine. The dihydro ester, on oxidation in ether solution with nitrous fumes or by iodine in alcohol solution, gives 2 : 6-dimethyl-4-ethylpyridine-3 : 5-dicarboxylate. The potassium salt obtained by hydrolysis when distilled with soda lime gives 2 : 6 dimethyl-4-ethylpyridine described by Engelmann. 2 : 6 Dimethyl-4-ethylpyridine has been condensed with benzaldehyde in presence of zinc chloride, when 2 : 6-distyryl-4-ethylpyridine, 2-styryl-4-ethyl-6-methylpyridine and α phenyl- β -6-(2-methyl-4-ethyl)-pyridyl-ethanol are formed. The monostyryl derivative gives a picrate, m.p. 232-33° but is formed in too small a quantity for detailed examination.

It has been mentioned (*J. Indian Chem. Soc.*, 1939, **16**, 399), that one of the basic products isolated by alkaline degradation of strychnine and brucine appeared to be 2-methyl-4-ethylpyridine from its physical constants. An attempt has been made to synthesise it by Hantzsch's general method for pyridines (*Annalen*, 1882, **216**, 1; *Ber.*, 1883, **16**, 1946; 1885, **18**, 1744-2579); Engelmann (*Annalen*, 1885, **231**, 44) obtained ethyl dihydroparvolinedicarboxylate by condensing a mixture of ethyl acetoacetate, propionaldehyde and alcoholic ammonia which on oxidation with nitrous acid gave ethylparvoline. It was hoped that it would give a monobenzylidine derivative with benzaldehyde, whence by oxidation and subsequent decarboxylation 2-methyl-4-ethylpyridine would be obtained.

2 : 6-Dimethyl-4-ethyl-1 : 4-dihydropyridine-3 : 5-dicarboxylate, prepared by a modified method (*vide* experimental) has m.p. 110° and gives 2 : 6-dimethyl-4-ethylpyridine-3 : 5-dicarboxylate on oxidation with iodine or nitrous acid in better yield in ether solution. The crude potassium salt obtained by hydrolysis with alcoholic potassium hydroxide has been mixed with soda lime and distilled. From the distillate 2 : 6-dimethyl-4-ethylpyridine has been obtained and when it is condensed with benzaldehyde in a sealed tube first at 140° and then at 180-85° in presence of zinc chloride, it gives the best yield of monostyryl product along with a distyryl derivative and a third substance, which from the analytical data, appears to be an aldol condensation product of the base and benzaldehyde. All the three products are formed irrespective of the proportions in which the reactants are present, but excess of benzaldehyde favours the formation of the distyryl product. The separation of the three products is effected by

taking advantage of the solubility of their salts. The monostyryl compound is not sufficient for further purification, detailed examination and characterisation. Further work is in progress.

EXPERIMENTAL.

2 : 6-Dimethyl-4-ethyl-1 : 4-dihydropyridine-3 : 5-dicarboxylate.—To a mixture of ethyl acetoacetate (115 g., 2 mol.) and propionaldehyde (25 g., 1 mol.) cooled in a freezing mixture was added cooled piperidine (16 g.). The mixture was kept in the freezing mixture for 2 hours and then allowed to stand overnight at 0°. Ammonia (a little more than 1 mol., 8 g.) was passed into the mixture and after the addition of a little more ammonia solution (*d* 0.880, 5 c.c.) was heated in a closed flask at 100° for 4 hours. After cooling the light yellow coloured mixture was decomposed with water when the dihydro ester separated in bright yellow flocks. It was well washed with hot water (yield 115 g., 89%). After crystallisation from ethanol in pale yellow prisms it had m.p. 112° (corr.) (*cf.* Engelmann, *loc. cit.*). The substance is soluble in ether, acetone, chloroform, ethyl acetate, benzene and alcohol but difficultly soluble in petroleum ether and insoluble in water. (Found in material dried at 100° in *vacuo* : C, 64.1 ; H, 8.1 ; N, 5.1. $C_{16}H_{23}O_4N$ requires C, 64.1 ; H, 8.2 ; N, 5.0 per cent).

The oxidation of the foregoing dihydro-ester with nitrous acid was best conducted in ether solution by passing nitrous fumes. After the removal of ether (washing of the ether solution with water to remove excess of nitrous acid was found undesirable) the residue was made alkaline with sodium carbonate and extracted with ether. After the removal of the solvent the residue was extracted with petroleum ether in which the original dihydro-ester was insoluble. The unchanged ester was resubmitted to oxidation. The petroleum ether solution was washed with water, dried over potassium hydroxide and allowed to stand overnight at 0° to remove traces of the dihydro-ester. The solvent was removed and the base distilled at 135-40°/0.5 mm. (pale yellow liquid).

The oxidation in ether solution gave much better yield than in alcohol. Oxidation was also tried with iodine in alcoholic solution. Dihydro-ester (4 g., 1 mol.) was added to a solution of iodine (3.6 g., 1 mol.) in alcohol (50 c.c.) and the solution was refluxed on the steam-bath for 1 hour. The solution was concentrated to 5 c.c. and after making alkaline with sodium carbonate was extracted with ether and the excess of iodine was removed by sulphur dioxide. The ester was isolated as hydrochloride whence the base was liberated with alkali and extracted with ether. The residue from ether was dissolved in petroleum ether when unchanged product (0.4 g.)

remained undissolved. The residue from petroleum ether was distilled at 135-140°/0.5 mm.

The *picrate* of 2:6-dimethyl-4-ethylpyridine-3,5-dicarboxylate was prepared from an ethereal solution of the base and picric acid in acetone when it separated as a mass of long lemon-yellow needles. After washing with water it was recrystallised from alcohol or water as hexagonal prisms, m.p. 116°. It is soluble in alcohol, acetone, benzene, chloroform and water, insoluble in ether and petroleum ether. It lost 3.4% H₂O at 100° (calc. for 1 H₂O, 3.4 per cent). [Found in material after drying at 100° in *vacuo*: C, 49.8; H, 5.0; N, 11.2. C₁₅H₂₁O₄N·C₆H₂(NO₂)₃(OH) requires C, 49.6; H, 4.7; N, 11.0 per cent].

The dihydro-ester was hydrolysed with alcoholic potassium hydroxide and the crude potassium salt (1 part) obtained by evaporation was mixed with soda lime (2 parts) and distilled in a tube sealed at one end. The base distilled at 184-86° to a straw-coloured fuming liquid with a strong odour, yield 90% (b.p. 186°, *cf.* Engelmann). The *picrate* of the base was obtained as bright yellow prisms and after recrystallisation from alcohol had m.p. 121° (Engelmann gives 119-120°). [Found in material dried at 100° in *vacuo*: C, 49.5; H, 4.4; N, 15.0. C₉H₁₃N·C₆H₂(NO₂)₃(OH) requires C, 49.5; H, 4.4; N, 15.4 per cent]. The *hydrochloride*, prepared by precipitation with hydrogen chloride of the ethereal solution of the base, was hygroscopic, pinkish long needles, m.p. 97°. It is soluble in alcohol, acetone, and water.

Condensation of 2:6-Dimethyl-4-ethylpyridine with Benzaldehyde.—A mixture of the base (7 g., 1 mol.), benzaldehyde (6.5 g., 1.2 mol.) and zinc chloride (1 g.) showing a green fluorescence was heated in a sealed tube first at 140° for 4 hours and then at 180-85° for 4 hours more. The resulting thick reddish liquid was made alkaline with sodium hydroxide and extracted with ether. The extract was washed with water and dried over potassium hydroxide. The residue from ether after being distilled in steam to remove benzaldehyde and unreacted base, was taken up in petroleum ether (40-60°). The solution after standing for some hours was filtered from a slight sediment and hydrogen chloride was led into the solution, when a sticky precipitate of the hydrochloride separated. It was dissolved in a little methanol and the solution precipitated by ether. The process was repeated two times and the filtrate combined, a small quantity of tarry matter being neglected. The clear solution showed a greenish blue fluorescence and on keeping at 0° deposited a light yellow crystalline mass of hydrochloride "A" (0.4 g.). The filtrate, on adding picric acid in acetone solution, gave bright yellow prisms, (*picrate* "B") a further yield of which was obtained by concentrating the solution (5 g.). The solids were

washed with hot acetone to remove any picrate of "A" which might have been present. Finally, the mother-liquor from the picrate was evaporated to dryness. The residue was triturated with sodium hydroxide solution. The resulting clear solution after the addition of water was then extracted with ether. The ether extract, after washing with water and drying over potassium carbonate, was treated with hydrogen chloride when an insoluble hydrochloride was precipitated. This was filtered and washed with ether. It was recrystallised by adding ether till turbidity to its methyl alcoholic solution and keeping at 0° when it separated in large white cubes (hydrochloride "C").

These experiments were conducted under varying conditions but in every case all the above three products were obtained as shown in the table below, even when only one molecule of benzaldehyde to one molecule of the base was used.

TABLE I.

Reactants	Temp	HCl of "A"	Picrate of "B"	HCl of "C"	Picrate of unreacted base.
(a) 1 Mol. base, 3 mol. benzaldehyde in an atmosphere of nitrogen	140°	0.6 g.	1 g.	2 g.	2 g.
(b) 1 Mol. base, 1 mol. benzaldehyde in a sealed tube.	140°	0.2	1	2.5	2.5
(c) 1 Mol. base, 1 mol. benzaldehyde in a sealed tube.	180° 190°	0.6	2.5	2	1.5
(d) 1 Mol. base, 1.2 mol. benzaldehyde in a sealed tube	140° and then 180° 185°	0.4	5	2	0.5

Fraction A.—2:6-Distyryl-4-ethylpyridine Hydrochloride.—The hydrochloride "A" on recrystallisation from methanol had m. p. $271-72^{\circ}$ (decomp.). In chloroform and alcohol solution it showed a blue fluorescence. [Found in material dried at 100° in *vacuo*: C, 78.9; H, 6.4; N, 4.4; Cl, 10.0. $C_{23}H_{21}N$, HCl requires C, 79.4; H, 6.3; N, 4.0; Cl, 10.2 per cent].

2:6-Distyryl-4-ethylpyridine Chloroplatinate.—The chloroplatinate was obtained in serrated prisms by adding an aqueous solution of platinic chloride to a solution of the hydrochloride in alcohol. It is insoluble in alcohol and water, m. p. 263° (decomp.). [Found in material dried at 100° in *vacuo*: C, 53.7; H, 4.3; Pt, 18.4. $(C_{23}H_{21}N, HCl)_2 \cdot PtCl_4$ requires C, 53.5; H, 4.3; Pt, 18.9 per cent].

The *aurichloride*, prepared in methanol solution with aqueous gold chloride, was obtained in golden yellow glistening plates. It is easily

soluble in chloroform, acetone and hot alcohol and insoluble in water, m. p. 200° .

α -Phenyl- β -6-(2-methyl-4-ethyl)-pyridyl-ethanol.—The base as obtained before converting it into the hydrochloride "C" was a thick syrupy brownish liquid. It is soluble in all the organic solvents and so far has not been obtained crystalline.

Fraction C.— α -Phenyl- β -6-(2-methyl-4-ethyl)-pyridyl-ethanol Hydrochloride.—The hydrochloride was obtained as above and on recrystallisation from alcohol-ether mixture it separated in perfectly white cubes. It is soluble in alcohol, chloroform, hot acetone and water, m. p. 175° . (Found in material dried at 100° in *vacuo*: C, 68.8, 68.9; H, 7.0, 6.9; Cl, 12.6. $C_{16}H_{19}ON \cdot HCl$ requires C, 69.2; H, 7.2; Cl, 12.7 per cent).

The *chloroplatinate*, prepared by adding an aqueous solution of platonic chloride to a methyl alcohol solution of the hydrochloride and diluting till turbidity and then keeping at 0° for a week, separated as an amorphous mass. It is soluble in alcohol, acetone, chloroform and water. It softens at 85° and gradually melts at 125° . The *picrate*, which came down as oily drops when an aqueous solution of picric acid was added to a methyl alcoholic solution of the hydrochloride, became crystalline on adding water to the solution and on scratching and standing it separated in prisms. It is easily soluble in alcohol, acetone, ethyl acetate, benzene and very sparingly in ether. It lost nothing on drying at 100° . [Found in dried material: C, 56.6; H, 4.5; N, 12.3. $C_{16}H_{19}ON \cdot C_6H_3(NO_2)_3(OH)$ requires C, 56.2; H, 4.7; N, 11.9 per cent].

Fraction B.—After purification as picrate it analysed for the picrate of monostyryl derivative. The quantity was not sufficient for further characterisation. The picrate had m. p. $232-33^{\circ}$. [Found in material dried at 100° in *vacuo*: C, 59.2; H, 4.8; N, 12.1. $C_{16}H_{17}N \cdot C_6H_3(NO_2)_3(OH)$ requires C, 58.4; H, 4.4; N, 12.4 per cent].

The author is highly grateful to Professor Sir Robert Robinson, Kt., F.R.S., for his advice and encouragement throughout this investigation and to the Trustees, Dawoodhbhoy Fazalbhoy Muslim Educational Trust, Bombay, for an Educational loan which enabled the author to take part in this investigation.

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Received July 21, 1939.

STUDIES IN THE PYRIDINE SERIES. PART II. A NEW SYNTHESIS OF 2-METHYL-4-ETHYLPYRIDINE.

BY RAFAT HUSAIN SIDDIQUI AND ABDUL QUDDUS KHAN.

The monostyryl base obtained from 2:6-dimethyl-4-ethylpyridine, has been oxidised to 2-methyl-4-ethylpyridine- α -carboxylic acid, which is decarboxylated to 2-methyl-4-ethylpyridine. The picrate of the latter, m.p. 142° , on admixture with the picrate of $C_8H_{11}N$ obtained after alkaline degradation of strychnine and brucine, melts at 125° thus proving the non-identity of the latter with 2-methyl-4-ethylpyridine.

In Part I (*J. Indian Chem. Soc.*, 1939, **16** 410) of this series a synthesis of 2-methyl-4-ethylpyridine was attempted by one of us (R.H.S.). By condensing 2:6-dimethyl-4-ethylpyridine and benzaldehyde in presence of zinc chloride an aldol condensation product, a distyryl and a monostyryl derivative have been obtained. In the present investigation acetic anhydride has been used instead of zinc chloride when the monostyryl (60%), the distyryl (25%) and some unreacted base (15%) have been isolated from the condensate and no trace of alkin is formed. The three bases have been separated *via* their salts (*vide* experimental). The distyryl hydrochloride is insoluble in water while the hydrochlorides of the monostyryl and the trialkyl base are easily soluble even in ice-cold water, the free bases being separated by distillation. After the removal of the trialkyl pyridine (b.p. $183-85^{\circ}$) the residue is distilled at $205^{\circ}/2$ mm. when the monostyryl derivative is obtained as a thick straw-coloured liquid. The base from distyryl hydrochloride crystallises from petroleum ether, m.p. 85° . The distyryl base does not condense with benzaldehyde further under the conditions tried. The monostyryl derivative (2-styryl-4-ethyl-6-methylpyridine) has been oxidised in acetone solution by means of potassium permanganate when benzoic acid and α -methyl- β -ethylpyridine- α' -carboxylic acid is obtained from the slimy precipitate while some unoxidised substance is recovered from the solvent. The α -carboxylic acid, on decarboxylation with a trace of copper powder, gives the desired base. This has been converted into a picrate, m.p. 142° . The m.p. of the picrate and its analytical values are in agreement with those given in literature for 2-methyl-4-ethylpyridine. This picrate on admixture with the picrate of the base $C_8H_{11}N$, isolated from strychnine and brucine as a result of alkaline degradation, shows a marked depression.

EXPERIMENTAL.

2:6 Dimethyl-4-ethyl pyridine (Part I, *loc. cit.*) was further purified *via* the picrate and then regenerating it from the picrate.

Condensation of 2:6-Dimethyl-4-ethylpyridine with Benzaldehyde.—A mixture of the base (1 mol., 12 g.), freshly distilled benzaldehyde (1.05 mol., 9.5 g.) and acetic anhydride (12 g.) were gently refluxed for 14 hours. The dark coloured solution was acidified with dilute hydrochloric acid and distilled in steam to remove benzaldehyde. The residue was again treated with more hydrochloric acid when some crystals "B" imbedded in a pasty mass separated from the acid solution "A". The crystalline mass "B" was washed with a little dilute acetic acid. The pasty mass "C" was dissolved in chloroform and after removal of tarry impurities by addition of ether to the solution it crystallised in hexagonal needles which proved identical with "B" (mixed m. p.) and was the hydrochloride of the distyryl derivative. The pasty residue from chloroform solution "C" was converted into a picrate and is under examination. The aqueous acid mother-liquor after cooling at 0° overnight was filtered from traces of the hydrochloride "B". The clear yellow filtrate was made alkaline with sodium hydroxide and shaken with petroleum ether. The petroleum ether solution was washed with water and dried over anhydrous sodium sulphate and after removal of the solvent gave a straw-coloured thick oily base. The unreacted base (2:6-dimethyl-4-ethylpyridine), b.p. 180-86°, was removed and identified by conversion into the picrate (m.p. 120°). The residue distilled at 205°/2 mm. to a thick oily liquid and proved to be 2-styryl-4-ethyl-6-methylpyridine, by conversion into the picrate, m.p. 232-33° (undepressed by that obtained from fraction B, cf. part I *loc. cit.*).

2-Styryl-4-ethyl-6-methylpyridine and its Salts.—The base, regenerated with alkali, was a straw-coloured oily liquid. It was converted into the hydrochloride. The sticky hydrochloride on washing with ether turned into a powder. To the well cooled yellow solution of the hydrochloride in water, a solution of potassium iodide in water was added when the hydroiodide of the monostyryl base came down as a sticky mass which soon turned to a mass of yellow needles. An attempt was made to crystallise the hydroiodide from dilute alcohol but it turned into an oily mass at ordinary temperature. It is soluble in common organic solvents. However, the monostyryl base in ether gave with ethereal hydrogen chloride a sticky hydrochloride. After washing with ether and triturating with a little acetone, it turned into a mass of needles with a pale tinge. It was soluble in alcohol, acetone, chloroform and water and had m.p. 208°. On drying in *vacuo* it suffered a loss of 32.7%. (Found in material dried at 100° in *vacuo*: C, 73.8; H, 6.55; N, 5.39. $C_{16}H_{17}N$, HCl requires C, 73.58; H, 7.25; N, 5.37 per cent).

The *hydroiodide*, prepared from the above hydrochloride, was crystallised from alcohol-ether mixture, m.p. 203° . It is soluble in alcohol, chloroform, acetone and water. It lost 4.2% of its weight at 100° in *vacuo*. (Found in dried material : C, 54.34 ; H, 4.90 ; N, 3.58 ; I, 36.95. $C_{16}H_{17}N$, HI requires C, 54.70 ; H, 5.13 ; N, 3.99 ; I, 36.19 per cent).

The *chloroplatinate*, prepared in the usual way, was obtained as an amorphous powder soluble in acetone, chloroform, alcohol and ethyl acetate, m.p. 243° after drying over P_2O_5 . [Found in anhydrous material : C, 45.57 ; H, 4.39 ; N, 3.0 ; Pt, 23.06 ($C_{16}H_{17}N$, HCl) $_2$. $PtCl_4$ requires C, 44.85 ; H, 4.20 ; N, 3.27 ; Pt, 22.8 per cent].

The *aurochloride* was obtained by mixing together aqueous solutions of gold chloride and the hydrochloride as golden yellow mass, m.p. 145° after drying. It is soluble in acetone and chloroform.

The *picrate* crystallised in lemon-yellow tufts of needles from acetone, m.p. 233° (sublimes at 90° in *vacuo*). [Found in material dried at 100° in *vacuo* : C, 58.63 ; H, 4.52 ; N, 12.35. $C_{16}H_{17}N$ $C_6H_3(NO_2)_3(OH)$ requires C, 58.41 ; H, 4.14 ; N, 12.39 per cent].

2 : 6-Distyryl-4-ethylpyridine and its Salts.

2 : 6-Distyryl-4-ethylpyridine.—The base from hydrochloride "B" dissolved in alcohol was isolated with aqueous sodium hydroxide solution and washed with water. Its solution in ether showed a violet fluorescence. The ether solution was washed with water and dried over anhydrous sodium sulphate. The residue from ether was dissolved in petroleum ether and filtered from a negligible quantity of tarry impurities. The light yellow coloured filtrate on keeping at 0° crystallised in bunches of closely packed small needles, m.p. 85° . It is soluble in alcohol, acetone, chloroform, benzene, ethyl acetate, ether and petroleum ether. At 100° in *vacuo* it lost 2.9% of its weight. (Found in material dried at 60° in *vacuo* : C, 88.19 ; H, 6.7 ; N, 4.53. Found in material dried at 100° in *vacuo* : C, 88.63 ; H, 6.95. $C_{23}H_{21}N$ requires C, 88.74 ; H, 6.75 ; N, 4.50 per cent).

The *hydrochloride* "B" crystallised in needles from dilute acetic acid. It may be prepared from the chloroform solution of the base with ethereal hydrogen chloride. On crystallisation from chloroform-alcohol mixture it separated in small light yellow needles, m.p. $271-72^{\circ}$. It is soluble in acetone, chloroform and methyl alcohol and difficultly in ethanol.

The *picrate*, prepared in ether solution, dissolved difficultly in alcohol and on concentrating the alcoholic solution crystallised in prisms, m.p. 255° . It is soluble in acetone. [Found in material dried at 100° in *vacuo* ;

C, 64.58; H, 4.28; N, 10.87. $C_{23}H_{21}N, C_8H_2(NO_2)_3(OH)$ requires C, 64.44; H, 4.44; N, 10.37 per cent).

Attempted Condensation of 2:6-Distyryl-4-ethylpyridine with Benzaldehyde.—When a mixture of 2:6-distyryl-4-ethylpyridine (1 mol., 6 g.), benzaldehyde (1.5 mol.; 3.5 g.) and acetic anhydride (6 g.) was heated on a sand-bath for 15 hours and the product worked out in the usual way only unchanged reactants were isolated in quantitative yield.

Oxidation of 2-Styryl-4-ethyl-6-methylpyridine: Formation of 4-Ethyl-6-methylpyridine-2-(a)-carboxylic Acid.—An ice-cold solution of styryl derivative (10 g.) in acetone (200 c.c.) was oxidised with finely powdered potassium permanganate (2.5 mols, 17 g.) added in portions during an interval of 30 minutes. The reaction mixture was filtered and the precipitate washed with acetone and extracted with hot water. The acetone solution gave some unreacted monostyryl derivative. The aqueous filtrate was acidified with hydrochloric acid and evaporated in *vacuo*. The residue dissolved in ether and after removal of the solvent, was taken up in alcohol and an alcoholic solution of lead acetate was added when a little lead salt (0.1 g.) separated which did not melt below 360°. The alcoholic filtrate was evaporated to dryness in *vacuo* and the residue was suspended in water and delead. The clear aqueous filtrate was again evaporated in *vacuo* and the residue was dissolved in petroleum ether and dried over anhydrous sodium sulphate. The solution after concentration was kept in ice-chest when some crystalline sediment appeared but it could not be separated and on filtering turned oily.

Decarboxylation of the Above acid.—The above acid was heated with a trace of copper powder when the base distilled with evolution of carbon dioxide.

2-Methyl-4-ethylpyridine Picrate.—The distillate was converted into the picrate as pale yellow needles, in ether solution, m.p. 142°, after washing with ether. It crystallised with $\frac{1}{2}$ mol. water of crystallisation which it lost at 100° in *vacuo*. (Found: H_2O , 3.0. Calc. 2.6 per cent). [Found in material dried at 100° in *vacuo*. C, 47.98; H, 3.97; N, 16.47. $C_8H_{11}N \cdot C_8H_2(NO_2)_3(OH)$ requires C, 47.99; H, 3.97; N, 16.0 per cent]. The m.p. of 2-methyl-4-ethylpyridine picrate as given in literature is 142°. The m.p. of synthetic picrate mixed with the picrate of the base $C_8H_{11}N$ obtained from alkaline degradation of strychnine and brucine, was indefinite (115-25°) thereby establishing non-identity.

The analyses were done by micro-method by Dr. Ing. A. Schöller, Berlin.

A RAPID METHOD OF ESTIMATING SMALL AMOUNT OF IRON AND MANGANESE IN COPPER-NICKEL-ZINC ALLOYS.

BY HARENDRA NATH ROY.

Estimation of small amount of iron (0.1 % max.) and manganese (0.3 % max) in alloys containing copper (60-80 %), nickel (15-20 %), and zinc (20 %) meets with some difficulty. It is generally done in the laboratory by precipitating the hydroxides of iron and manganese by ammonia from a nitric acid solution of the alloys and precipitating it again so as to make it as much free from copper, nickel and zinc as possible. It is then ignited and weighed. The ignited oxides are dissolved in a few drops of hydrochloric acid and the latter is removed by nitric acid. Manganese is estimated by silver nitrate-persulphate method and the iron calculated by difference.

By this process the above precipitate cannot be made completely free from copper, nickel and zinc. Direct determination of iron by titanous sulphate, though rapid, suffers from the defect that the solution cannot be preserved unchanged for any length of time, as the strength of it is to be kept very weak (1 c.c. commercial TiSO_4 diluted upto 100 c.c. or even more).

The following method is suggested as an improvement. Iron is first determined by reducing the solution with metallic zinc and then titrating it with $N/50\text{-KMnO}_4$. Manganese is afterwards estimated by the ordinary arsenite method. As the amount of additional manganese, which has been introduced into the solution while titrating iron, is known, it is deducted from the final manganese content of the alloy.

Procedure.

The alloy (1 g.) is dissolved in 20 c.c. HNO_3 (d 1.2) and diluted to 100 c.c. with water. Bromine water (10-15 c.c.) is added and made ammoniacal. Mn and Fe are precipitated down as hydroxides; Cu, Ni and Zn remain in the solution. It is filtered and washed alternately 5 to 6 times with hot water and ammonia. A solution of sodium metabisulphite (5 %) containing H_2SO_4 (1.20) is freshly prepared and the precipitate is dissolved by adding this solution drop by drop. The solution is received in a conical flask, 5 to 6 c.c. concentrated H_2SO_4 are added together with 10 c.c. of 10% solution of ammonium persulphate. If any

sulphur separates out at this stage, it is oxidised by the persulphate on boiling. The solution is boiled for 5 minutes until the persulphate is completely decomposed. The solution is cooled to 60° , the iron is reduced in a Jone's apparatus and titrated against $N/50\text{-KMnO}_4$.

1 C.c. of $N/50\text{-KMnO}_4$ contains 0.00112% iron.

After the titration of iron the same solution is used for the determination of manganese.

A 0.5% solution (20 c.c.) of AgNO_3 (according to the percentage of manganese present) is introduced into the flask containing the solution for the estimation of iron and is heated to boiling. A 5% solution of ammonium persulphate (20 c.c.) is added to the boiling solution when the permanganate colour is developed. It is then cooled in running water. Finally $N/50$ -sodium arsenite solution is run in till the colour of the permanganate is discharged.

The sodium arsenite is standardised against a standard steel of known manganese content.

Sample No.	Iron present.	Iron found.	Total Mn. found.	Mn found after-deducting the Mn in $N/50\text{ KMnO}_4$.	Actual Mn. present.
1.	0.09%	0.09%	0.219%	0.20%	0.20%
2.	0.08	0.079	0.228	0.21	0.21
3.	0.10	0.098	0.209	0.19	0.195

CALCUTTA.

Received January 30, 1939.

A NOTE ON THE ALKALOIDS OF *RAUWOLFIA* *SERPENTINA*, BENTH.

BY SALIMUZZAMAN SIDDIQUI.

The investigation of the alkaloidal constituents of the *Rauwolfia Serpentina* roots, collected from the Bihar province, had led to the isolation of a series of new alkaloids, namely ajmaline, $C_{20}H_{26}O_3N_2$ (m.p. $158-60^\circ$), ajmalinine, $C_{20}H_{26}O_3N_2$ (m.p. 181°), and ajmalicine (m.p. 252°) which are white crystalline bases and serpentine, $C_{20}H_{20}O_3N_2$ (m.p. 158°) and serpentinine, $C_{20}H_{26}O_3N_2$ (m.p. 265°) which are bright yellow crystalline products. The mutual relationship of four of these alkaloids has been fully discussed (Siddiqui and Siddiqui, *J. Indian Chem. Soc.*, 1935, 10, 37 and preceding papers).

Since then further work on the constitution of these alkaloids had to be postponed owing to the difficulty of obtaining fresh air-dried roots and extremely poor yields of the bases from carelessly collected and stocked samples of the drug. Recently the author made a systematic study of the alkaloidal constituents of the roots and root-bark obtained from the Dun valley.

The detailed results of this investigation will be communicated later. In the present note it is merely intended to report that the constituents of the Dun drug are allied to the ajmaline series but are different in yield and character from alkaloids isolated from the Bihar variety. Thus, no ajmaline could be isolated from the crystalline hydrochloride fraction, and only traces of ajmalinine and serpentinine were found in one of the samples. On the other hand, isoajmaline (m.p. $264-66^\circ$) and an apparently new isomer, which has been provisionally named neoajmaline, (m.p. $205-7^\circ$), and which can be converted on heating at 270° or with alcoholic potash, into isoajmaline, were isolated from the crystalline hydrochloride, in 0.1 and 1% yields respectively from the root-bark and 0.01% and 0.1% yields from the whole roots. Another new alkaloid of this group melting at 220° has also been isolated from the root-bark and whole roots (yield about 0.02%) out of the alcohol-insoluble fraction of the base obtained from the mother-liquors of the crystalline hydrochloride. Further, from the neutral fraction of the alcoholic extract, a white crystalline alkaloid of amphoteric character melting at 234° was isolated in a yield of 0.1%.

Altogether the Dchradun variety contains very little of the yellow group of bases which are obviously oxidation products of the white ajmaline group. It is thus seen that the yellow oxidation bases of the plant growing in the hot swampy districts of Bihar are not formed in the milder climatic conditions of the Dun valley. It is interesting to note this from the point of view of the genetic connection between the two groups of bases in the plant body and the possibility of controlling the yield of one or the other of these groups by regional choice for the growth of the plant. The therapeutic importance, which the ajmaline group of alkaloids has gained, as a valuable hypnotic and general sedative for nervous complaints and for their curative properties in certain types of mental disorder, lends special significance to the findings reported in this note.

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Received August 16, 1939.

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THE CONSTITUENTS OF *DIDYMOCARPUS PEDICELLATA*.
PART III. ISOLATION OF A SESQUITERPENE AND
TWO POLYTERPENE PRODUCTS AND EXA-
MINATIO . OF THE FATTY MATTER.

BY SHARIFUDDIN WARSI AND SALIMUZZAMAN SIDDIQUI.

A sesquiterpene, didymocarpene, has been isolated from the essential oil of the leaves. From the heavier essential oil fraction two terpenic products, didymocarpol ($C_{50}H_{100}O_5$) and didymocarpenol ($C_{25}H_{42}O$) have been isolated and characterised. These two products have also been isolated from the unsaponifiable matter in the non-volatile, fatty residue. The saturated saponification acids are found to consist of palmitic, stearic, behenic and lignoceric acids in nearly equal proportions. Free stearic acid is also found to be present in the leaves.

The process of isolation of pedicin and three other allied colouring matters (Siddiqui, *J. Indian Chem. Soc.*, 1937, **12**, 703) from the ethereal extract of the leaves of *Didymocarpus pedicellata* entailed the simultaneous separation of (a) an essential oil, (b) a neutral, and (c) an acid fraction. The present paper deals with the investigation of these three fractions, which together form the main bulk of the total ethereal extract and from which the following products have now been isolated.

1. A sesquiterpene, didymocarpene, $C_{15}H_{24}$.
2. Didymocarpol, $C_{50}H_{100}O_5$, m.p. 76° .
3. Didymocarpenol, $C_{25}H_{42}O$ m.p. 137° .

Of these three products the sesquiterpene is obtained through repeated fractional distillation of the lighter essential oil which distilled over on passing a moderate current of steam through the petroleum ether-soluble neutral portion of the ethereal extract. The two polyterpenes are obtained from the heavier essential oil, which later distill over in a vigorous current of steam. These terpenes are also obtained from the unsaponifiable fraction of the fatty residue, left after steam distillation.

Didymocarpene does not yield a crystalline nitrosyl chloride or nitrosate but may be characterised through the formation with nitrous acid of a crystalline product, m.p. $132-34^\circ$, which appears to be a nitroso-bisnitrosite having the formula $C_{15}H_{22}NO \cdot (ON \cdot NO)_2 \cdot C_{15}H_{24}$. Titration with bromine in the cold points to the presence of two double bonds in the molecule. Apparently didymocarpene belongs to the class of bicyclic sesquiterpenes.

The specific gravity and refractive index of didymocarpene also approximate closely to values required for a bicyclic mono-terpene. Didymocarpol is optically inactive, saturated to bromine, rather inert and stable to alkaline or oxidative degradation. Didymocarpenol contains a single double bond and is strongly *laevorotatory*.

The saturated acids obtained by saponification of the fatty residue appear to consist of lignoceric, behenic, stearic and palmitic acids in nearly equal proportions. Of these the two latter are isolated by repeated fractional crystallisation from dilute alcohol but the former two form an unseparable eutectic mixture whose composition is, however, indicated by its m.p. (75-76°), C-H values and equivalent weight. The unsaturated acids are not further investigated.

Stearic acid was also isolated from the acidic fraction of the original ether extract. None of the other saturated acids could be noted in the free fatty acid fraction.

EXPERIMENTAL.

Isolation of Didymocarpene.—The light greenish yellow oil, obtained on steam distillation of the final filtrates from pedicellin (Siddiqui, *loc. cit.*) was distilled under reduced pressure, when the following fractions were obtained :—

- (1) 75-130°/4 mm., 5 g. of an oil smelling of benzaldehyde.
- (2) 132-40°/4 mm., 44 g. of an odorous straw-coloured oil.
- (3) 140-45°/4 mm., 10 g. deep yellow oil.
- (4) 145-50°/4 mm., 8 g. dark oil.

The residue (2 g.) was rejected.

The benzaldehydic smell of the first fraction was apparently due to contamination with traces of benzaldehyde, produced through hydrolytic splitting of the residual colouring matter, during steam distillation. On redistillation of this fraction and fractions (3) and (4), nearly 20 g. more of fraction (2) were obtained. The combined fractions (2) on rectification finally yielded 64 g. of pure didymocarpene slowly distilling at 135-37°/10 mm. (yield about 1·6% calculated on the weight of the dry leaves).

Didymocarpene is a thin straw-coloured oil with a characteristic pleasant smell. It boils at 136-37°/3 mm. and 147-48°/12 mm. and the b.p. greatly fluctuates with the rate of heating. It showed $[\alpha]_D^{25}, -3\cdot7^\circ$ in 1% absolute alcoholic solution; $d_4^{25}, 0\cdot8957$; $n_D^{25}, 1\cdot4988$. [Found: C, 87·9; H, 12·0; M.W. (cryoscopic in benzene), 188. $C_{15}H_{24}$ requires C, 88·2; H, 11·8 per cent. M. W., 204).

Action of Nitrous Acid on Didymocarpene: Didymocarpene-nitroso-bis-nitrosite.—Glacial acetic acid (3 c.c.) was added drop by drop to an ice-cooled mixture of 1 c.c. of oil in petroleum ether and 1 c.c. of concentrated aqueous sodium nitrite. After addition of the acid the bluish coloured mixture was allowed to stand for 2 hours, when the blue colour deepened. The petroleum ether layer was then separated, washed well with water, dried over sodium sulphate and the solvent removed. The dark bluish green residue was dissolved in a small quantity of absolute alcohol and kept in the cold, when it yielded sky-blue prismatic rods which after a few recrystallisations from the same solvent finally melted at $132-34^{\circ}$. (Found after drying to constant weight at 50° in *vacuo* over P_2O_5 : C, 63.45; H, 8.7; N, 12.1. $C_{30}H_{47}O_5N_5$ requires C, 62.8; H, 8.3; N, 12.5 per cent).

Action of Bromine on Didymocarpene.—0.49 G. of the substance in chloroform was titrated in the cold with a 3% solution of bromine in chloroform. The absorption of bromine, which was followed with the help of starch iodide paper, stopped after 0.66 g. (3.4 atoms equivalent of bromine) had been added.

Isolation of Didymocarpol and Didymocarpenol.

The residue left after steam distillation of didymocarpene was subsequently subjected to direct heating in a vigorous current of steam. The thick reddish yellow oil which distilled over was taken up in ether, washed, dried and distilled under reduced pressure after removal of the solvent, when the following fractions were obtained.

- | | | |
|----------|---|--|
| Fraction | 1 | up to $150^{\circ}/2$ mm., a very small quantity of crude didymocarpene. |
| „ | 2 | $155-95^{\circ}/2$ mm. a yellowish red oil (16 g.) |
| „ | 3 | $195-250^{\circ}/2$ mm., a dark red oil (8 g.) |

The residue was rejected. Fractions (2) and (3) crystallised on standing.

Didymocarpol ($C_{10}H_{20}O$)₅.—After a few crystallisations from alcohol the crystalline mass from fraction (2) was finally obtained as white silky needles, m. p. 76° (yield 0.3% calculated on the weight of the dry leaves). It failed to give an acetyl, benzoyl or a phenylhydrazine derivative and was saturated to bromine. It was stable towards potassium permanganate and could be recovered unchanged after refluxing with alcoholic potash at $140-50^{\circ}$ for 2 hours. (Found after drying at 50° in *vacuo* over P_2O_5 : C, 76.3; H, 12.8; M.W., 750. $C_{50}H_{100}O_5$ requires C, 76.9; H, 12.8 per cent. M. W., 780).

Didymocarpenol, $C_{25}H_{42}O$.—Fraction (3) also yielded after a few recrystallisations from alcohol snow-white silky needles, m.p. 137° ,

yield 0.15%. It failed to give any acetyl, benzoyl or phenylhydrazoné derivative but absorbed two atoms equivalent of bromine in chloroform solution. [Found after drying to constant weight at 100° in *vacuo* over P_2O_5 : C, 83.6; H, 12.0; M. W. (Rasl-), 376. $C_{28}H_{42}O$ requires C, 83.8; H, 11.7 per cent. M. W., 358]. In 1% absolute alcoholic solution it showed $[\alpha]_D^{20}, -65.5^\circ$.

Examination of the Non-volatile Fatty Residue.

Half of the residue, non-volatile in steam, was taken up in ether and shaken with dilute sodium hydroxide. The alkali soluble portion was taken along with the alkali-soluble ethereal mother liquors from pedicin. These together yielded stearic acid, m.p. 69° yield 8 g.

The neutral fraction deposited another crop of pedicellin which was filtered off. The filtrate was freed from the solvent and the residue saponified with 20% alcoholic potash. The acids were separated into the saturated and unsaturated fractions by the lead salt method.

The saturated acid yielded on repeated fractional crystallisation from dilute alcohol 5.5 g. (11% total fatty) of a fraction melting at 75-76°. (Found after drying to constant weight at 50° in *vacuo*: C, 77.6; H, 12.9; Equiv., 351. $C_{23}H_{46}O_2$ requires C, 77.9; H, 12.9 per cent. Equiv., 355).

The residue from the mother-liquors yielded on further fractional crystallisation 2.7 g. of pure stearic acid (m.p. 69°). The most soluble fraction gave 2.3 g. of palmitic acid, m.p. 60-62°. Both these acids showed no depression in m.p. on admixture with authentic specimens of the respective acids.

The unsaponifiable matter yielded further quantities of didymocarpol and didymocarpenol on distillation under reduced pressure and subsequent crystallisations of the respective fractions from alcohol.

CALORIFIC VALUE OF INDIAN FOODSTUFFS.

BY K. P. BASU AND M. C. MALAKAR.

The calorific value of about one hundred common foodstuffs has been determined by the Benedict-Fox oxycalorimeter. Moisture and nitrogen contents of the foodstuffs have also been determined.

The energy requirement of adults at rest and pursuing various activities, of children and of pregnant and lactating women has been determined and an expert commission of the League of Nations has made definite recommendations about this.

The object of the present investigation was to determine the potential energy value of foodstuffs we commonly use. In America, Sherman, M. S. Rose, Benedict *et al* and others have determined the calorific value of foodstuffs used in their country and from their data it is easy to calculate whether the amount of food taken by a man or a family is sufficient or not as regards the intake of energy.

Method of Determination.

In arriving at the energy value of any given diet it is customary to burn weighed samples of the various foods in an oxygen atmosphere in a bomb-calorimeter.

The complexity of manipulation and calculation and the high initial cost of the bomb calorimeter are absent in the "Oxycalorimeter", an apparatus developed by Benedict and Fox (*Ind. Eng. Chem.*, 1925, **17**, 912) in the Nutrition Laboratory of the Carnegie Institution of Washington, Boston. The principle involved in the oxycalorimeter is the direct determination of the volume of oxygen required to burn a known weight of a substance, and the computation therefrom of the potential energy of the substance, based upon a series of factors for the calorific value of a litre of oxygen previously established by the combustion of similar material in a bomb calorimeter.

There is another method of determining the energy value of foodstuffs which consists in determining the amounts of carbohydrates, fats and proteins in the food. We know the energy value of carbohydrates, fats and proteins and thereby can calculate the total energy content. But this method requires time and tedious chemical analysis.

E X P E R I M E N T A L.

In this investigation the energy value of different foodstuffs has been determined by the Benedict-Fox oxy-calorimeter.

After combustion the crucible was weighed, the unburnt carbon was heated over a Bunsen flame and again weighed to a constant value. Thus the amount of unburnt carbon was determined and a correction was made, 1.9 c.c. of oxygen being added for each mg. of carbon.

In the oxidation of a substance containing nitrogen, free nitrogen was liberated. Accordingly when such a combustion takes place in a confined volume of oxygen, the contraction as measured will always be materially less than that represented by the true volume of oxygen absorbed in the oxidation. Nitrogen content of the samples was determined by Kjeldahl's method and a correction was made.

The foodstuffs were collected from the local market and a representative sample of each foodstuff was prepared. In the case of vegetables and fruits, only the edible portions were taken, dried and water-content determined. First of all the volume corresponding to each millimeter length of the graduation of the scale in the apparatus was ascertained. Then the apparatus was standardised by burning a substance of known purity (sucrose). Pure sucrose having a heat of combustion of 3.949 calories per g. required in the oxy-calorimeter 783 c.c. of oxygen for the combustion of 1 g. The heat of combustion, 3.949 calories, divided by the oxygen consumption per g., 783 c.c., gives 5.04 calories per litre of oxygen, a factor exactly the same as that computed from the chemical equation for oxidation.

The weighed and dried samples of the foodstuffs were then burnt in the apparatus. Volume of oxygen required to burn was determined at N. T. P., correction for unburnt carbon and for the amount of nitrogen being made.

Benedict gives the calorific value of one litre of oxygen in burning a substance of high carbohydrate nature as 5.04 calories, for a substance with high fat content 4.70, and for a substance of high nitrogen content 4.68 calories. But in calculations in this paper calorific value of 1 litre of oxygen at N. T. P. has been taken to be 4.825 calories (kilocalories). "If one considers the average food of man and realises that in determining the metabolism of humans from the oxygen measurements the calorific value of a litre of oxygen is commonly taken as 4.825, one can see that this average values would not be far from correct for all food mixtures, particularly if a composite sample of the total daily meals were taken" (Benedict and Fox, *loc. cit.*).

Results of the investigation are given in the following tables.

TABLE I.

Bengali name.	Botanical name.	Wt. of sample.	Moisture	N ₂ by wt.	Unburnt C.	O ₂ absorbed at N. T. P. (corr. for Fe wire).	C. added for C.	C. added for N ₂ .	Total vol. of O ₂ absorbed	Caloric value of the sample.	Calories per 100 g.
Rice (Balam)	<i>Oryza sativa</i>	1.0345g.	9.8%	1.52%	0.067g.	636.5 c.c.	127.3	12.64	776.44	3.745	361.9
Balam (old)	"	1.01	10.1	1.48	0.02	789.7	38.0	11.8	839.5	4.046	400.5
Malati (sun-dried)	"	1.0003	10.4	1.42	0.064	531.0	121.6	11.36	663.96	3.203	319.5
Malati (par-boiled)	"	0.9988	10.0	1.38	0.042	528.3	79.8	11.04	619.14	2.986	299.0
Kataribhog (sun-dried)	"	1.0326	10.3	1.27	0.081	576.9	153.9	10.16	740.96	3.574	346.0
Kataribhog (par-boiled)	"	1.0094	8.6	1.24	0.06	550.5	114.0	9.92	674.42	3.254	322.5
Chinigura	"	1.0049	10.8	1.22	0.057	480.5	108.3	9.76	598.56	2.887	287.4
Nolbhog	"	1.0088	10.6	1.46	0.05	526.3	95.0	11.68	632.98	3.054	280.7
Swapan jhuri	"	1.0107	10.8	1.14	0.10	500.1	190.0	9.12	699.22	3.373	333.6
Bak-tulasi	"	1.0092	11.2	1.008	0.06	469.1	114.0	8.06	581.16	2.803	277.9
Sashi balam	"	1.2576	10.9	1.04	0.05	588.3	95.0	10.8	694.1	3.349	266.3
Chengar bhusi (par-boiled)	"	1.1216	9.08	1.38	0.052	510.3	98.8	12.0	621.1	2.994	266.9
Chengar bhusi (sun-dried)	"	1.0262	11.2	1.71	0.10	501.0	190.0	13.68	704.68	3.399	331.3
Kala jira	"	1.0494	12.3	1.135	0.066	519.1	125.4	9.04	653.54	3.152	300.4
Aman (old)	"	1.0434	10.7	1.305	0.021	846.5	39.9	10.4	896.8	4.326	414.8
Aus	"	1.0046	11.37	1.3	0.09	562.6	171.0	10.4	744.0	3.589	357.2

TABLE I (contd.).

Bengali name.	Botanical name.	Wt of sample.	Moisture.	N ₂ by wt.	Unburnt C.	O ₂ absorbed at N. T. P. (cor for Fe wire)	C added for C	C.c., added for N ₂	Total vol of O ₂ absorbed.	Calorific value of the sample	Calories per 100 g.
Abchaya (Ans)	<i>Oryza sativa</i>	1.0 g.	11.4	1.5	0.08	576.9	152.0	12.0	740.9	3.74	357.4
Kshud rice embryo)	"	1.0316	12.0	1.6	0.10	449.1	190.0	12.8	651.9	3.145	304.7
Kura (rice polishing)	"	1.0064	6.8	1.5	0.08	411.3	152.0	12.0	575.3	2.77	275.4
Muri (fried rice)	"	1.0014	3.8	1.4	0.08	501.3	152.0	11.2	664.5	3.20	319.7
Khoi (puffed rice)	"	1.0006	7.6	1.22	0.12	466.1	228.0	9.76	713.86	3.44	343.8
Binni (fried paddy)	"	1.0184	7.4	1.00	0.12	561.9	228.0	8.0	797.9	3.85	378.3
Chira (beaten rice)	"	1.0120	9.9	1.8	0.07	466.9	113.0	14.4	614.3	3.01	294.9
Mayda (wheat flour polished)	<i>Triticum vulgare</i>	1.0046	11.2	1.94	0.08	586.3	152.0	13.12	751.42	3.62	360.2
Ata (whole wheat flour)	"	1.0018	11.7	1.95	0.04	545.9	76.0	16.0	637.9	3.98	307.4
Suji	"	1.007	12.2	1.6	0.06	551.8	114.0	12.8	678.6	3.27	324.7
Barley	<i>Hordeum vulgare</i>	1.0266	2.5	1.77	0.14	466.1	266.0	14.16	746.26	3.6	350.5
Arrowroot	<i>Maranta arundinacea</i>	1.0038	11.6	0.10	0.10	538.4	190.0	0.80	729.2	3.52	350.5
Sago	<i>Metroxylon sago</i>	1.0044	7.6	0	0.004	686.9	7.6	0	694.5	3.35	333.6
Jaber chatu	"	1.0048	6.7	1.9	0.10	482.7	190.0	15.2	687.9	3.32	330.4
Pulses.											
Masuri (lentil)	<i>Lens esculenta</i>	1.0214	9.5	4.6	0.08	577.1	152.0	36.8	765.9	3.695	361.9
Khesari	<i>Lathyrus sativa</i>	1.1804	10.1	5.31	0.088	593.0	167.2	50.4	810.6	3.91	328.9
Bara motor (field pea)	<i>Pisum sativum</i>	1.1120	9.1	4.66	0.06	502.1	114.0	40.8	656.9	3.17	285.1
Choto motor	"	1.0028	10.1	4.91	0.05	502.1	95.0	40.0	637.1	3.07	336.4
Mung	<i>Phaseolus mungo</i>	1.0094	7.3	4.33	0.06	609.5	114.0	34.4	757.9	3.66	362.8
Chhola (horse gram)	<i>Cicer arietinum</i>	1.0047	9.38	3.84	0.07	640.4	113.0	30.4	783.8	3.78	376.1
Maskali (Black gram)	<i>Phaseolus radiatus</i>	1.0011	7.8	4.51	0.07	567.2	113.0	36.0	716.2	3.45	344.6
Arhar	<i>Cauphus indicus</i>	1.0129	10.2	3.83	0.09	486.6	171.0	30.4	688.0	3.32	328.0
Dabri (field bean)	<i>Dolichos lablab</i>	1.0156	9.8	4.075	0.054	515.8	107.6	32.0	655.4	3.16	311.1
Bean	"	1.0036	10.4	5.29	0.058	569.4	110.2	44.22	723.82	3.49	347.6

TABLE I (contd.).

Bengali name.	Botanical name.	Dry weight of sample.	Corresponding raw wt. of the sample.	Moisture.	N ₂ (on dry sample) %	Unburnt C.	Vol of O ₂ absorbed at N.T.P. (cor. for Fe wire).	C. added for C.	C. added for N ₂	Total vol. of O ₂ absorbed.	Calorific value of sample.	Calories per 100g (raw wt.).
Vegetables.												
Begun (Brinjal)	<i>Solanum melongena</i>	1.03148	10.97 g.	90.57%	1.75%	0.0378	370.3	70.3	11.2	451.8	2.18	19.63
Potato	<i>Solanum tuberosum</i>	1.0124	5.087	80.07	2.6	0.02	619.0	38.0	20.8	677.8	3.27	64.27
Misti kumra (pumpkin)	<i>Cucurbita maxima</i>	1.0268	12.08	91.51	2.23	0.05	344.9	95.0	17.84	457.74	2.21	18.3
Mula (radish)	<i>Raphanus sativus</i>	1.0008	19.41	94.81	1.54	0.09	308.0	171.0	12.32	491.32	2.37	12.21
Misti ala (sweet potato)	<i>Ipomoea batatas</i>	1.0296	3.3868	69.6	0.65	0.013	747.5	24.7	5.6	777.8	3.75	110.8
Kancha kala	<i>Musa paradisiaca</i>	1.0186	13.2285	92.3	0.96	0.02	655.7	38.0	7.7	691.4	3.33	25.17
Kancha penpe	<i>Carica papaya</i>	1.0108	12.635	92.0	1.54	0.041	333.1	77.9	12.32	423.3	2.04	16.14
Ada (ginger)	<i>Zingiber officinale</i>	1.0475	13.604	92.3	2.28	0.08	255.5	152.0	18.2	445.74	2.05	15.08
Sasha (cucumber)	<i>Cucumis sativus</i>	1.0786	24.2357	97.2	1.55	0.04	133.8	76.0	8.4	218.2	1.05	0.433
Fulkopi (cauli flower)	<i>Brassica oleracea botrytes</i>	1.0222	12.0963	91.7	4.53	0.08	461.6	152.0	36.0	649.6	3.13	25.86
Shalgum (turnip)	<i>Brassica rapa</i>	1.0246	12.6185	91.9	1.2	0.14	271.8	266.0	9.6	547.4	2.64	20.91
Beet	<i>Beta vulgaris</i>	1.0746	8.203	86.9	2.6	0.09	253.9	171.0	20.8	445.7	2.15	26.2
Ol kapi (knol khol)	<i>Brassica oleracea caulorapa</i>	1.0276	14.68	93.0	3.12	0.10	216.5	100.0	24.96	431.56	2.08	14.17
Gajar (carrot)	<i>Daucus carota</i>	1.0696	9.99	89.31	1.4	0.10	234.9	190.0	11.2	436.1	2.1	21.02
Sheem (cluster beans)	<i>Cyamopsis psoralioides</i>	1.0538	10.231	89.7	3.0	0.10	327.2	190.0	24.0	514.2	2.48	24.25
Karela (bitter gourd)	<i>Momordica charantia</i>	1.0604	12.9317	91.8	3.13	0.08	271.8	152.0	25.04	448.84	2.16	16.7
Palong sak	<i>Spinacia oleracea</i>	1.1042	15.7742	93.0	4.27	0.11	271.8	209.0	34.16	514.96	2.48	15.73
Garlic	<i>Allium sativum</i>	1.0104	2.9718	66.0	3.3	0.04	600.3	76.0	16.4	702.7	3.4	114.4
Onion	<i>Allium cepa</i>	1.10	6.25	84.2	1.33	0.026	581.9	49.4	10.6	641.9	3.1	49.61
Tomato (green)	<i>Lycopersicon esculentum</i>	1.0234	19.3094	94.7	1.7	0.06	218.9	114.0	13.6	346.5	1.67	8.64
Betel leaf	<i>Piper betel</i>	1.082	6.5575	83.5	2.21	0.10	560.7	190.0	17.7	768.4	3.71	56.58
Lettuce	<i>Lactuca sativa</i>	1.034	11.4888	91.0	3.26	0.108	280.3	205.2	26.0	511.5	2.47	21.49
Data	<i>Amaranthus gangeticus</i>	1.007	12.884	95.6	3.8	0.05	127.0	95.0	22.4	244.4	1.18	9.16
Sak ala	<i>Pachyrhizus angulatus</i>	1.028	6.5286	84.3	1.2	0.05	255.0	95.0	9.6	359.6	1.73	26.50

TABLE I (contd.).

Bengali name.	Botanical name.	Dry weight of sample.	Corresponding raw wt of the sample.	Moisture.	N ₂ (on dry wt).	Unburnt C.	Vol of O ₂ absorbed at N.T.P. (cor for Fe wire).	C. added for unburnt C.	C. added for N ₂ .	Total vol of O ₂ absorbed.	Calorific value of the sample.	Calories per 100 g (raw wt.).
Fruits.												
Khajur (Date palm)	<i>Phoenix dactylifera</i>	1.0204	1.2244	16.7%	0.55%	0.5 g.	634.8	95.0	4.4	734.2	3.54	289.2
Kissmis grape.	<i>Citrus grandis</i>	0.9101	1.1712	22.3	0.71	0.08	506.9	152.0	5.7	664.6	3.21	274.1
Apple	<i>Pyrus malus</i>	1.1206	7.8216	85.2	0	0.06	724.7	114.0	0	838.7	4.046	51.72
Neshpati		1.0028	8.3566	88.0	0.25	0.004	863.0	7.6	2.0	873.2	4.21	50.39
Badam (almond)	<i>Prunus cernigolia</i>	1.086	1.1821	8.1	4.0	0.004	1521.0	7.6	32.0	1560.6	7.53	637.1
Coconut	<i>Cocos nucifera</i>	1.04	2.0	48.0	1.4	0.005	1484.0	9.5	11.2	1504.7	7.26	363.0
Bangec		1.0066	4.19416	97.6	1.25	0.11	274.9	209.0	10.0	493.9	2.382	5.68
Bel		1.00	2.7154	61.7	0.93	0.01	655.1	19.0	7.4	681.5	3.3	121.5
Olive		1.0332	7.9476	87.0	0.76	0.02	626.9	38.0	6.7	671.0	3.24	40.77
Banana (ripe)	<i>Musa paradistaca</i>	1.0	3.9215	74.5	0.87	0.024	868.4	45.6	6.7	920.7	4.44	113.2
Egg white (Duck's)		1.0338	7.9523	87.0	13.7	0.002	1091.0	3.8	109.6	1204.4	5.8	72.93
Egg white (Hen's)		1.0646	7.7707	86.3	14.3	0.05	961.3	95.0	114.4	1170.7	5.65	72.71
Egg yellow (Duck's)		1.024	2.0157	49.2	5.28	0.04	1542.0	70.0	42.2	1660.2	8.0	396.8
Egg yellow (Hen's)		1.0506	2.144	51.0	5.44	0.048	1506.0	61.2	43.5	1640.7	7.92	378.0
Chhana (Milk protein)		1.0068	2.9875	66.3	6.82	0.03	1346.0	57.0	54.5	1457.5	7.03	235.4
Dry milk (whole)		1.0506		5.0	4.44	0.02	843.6	144.0	32.0	1019.6	4.92	489.4

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Received August 28, 1939.

CÖ-ORDINÄTED COPPER COMPOUNDS WITH PROPYLENEDIAMINE.

BY PANCHANAN NEOGI AND KANAI LAL MANDOL.

Literature shows that complex propylenediamine salts of copper were unknown. In our attempts to prepare *tris*-propylenediamine salts with a view to their resolution into optical isomers we have discovered *bis*-propylenediamine salts which were not resolvable. These salts are violet in colour, very soluble in water yielding beautiful violet coloured solutions. The *d*-tartrate, *d*-camphor sulphonate and nitronate have been prepared and their rotations measured.

Literature shows that copper forms complex ethylenediamine salts, but complex propylenediamine salts of copper were hitherto unknown. *bis*-Propylenediamine salts were however obtained by adding two molecular proportions of propylenediamine to aqueous solutions of soluble copper salts. All the solid salts are stable in air and possess beautiful violet colour, sulphate being bluish violet. They are extremely soluble in water and yield violet coloured solutions of different shades. The salts are not acted upon by dilute caustic alkalis, but acids, and even dilute acetic acid, decompose them as in the case of complex ammonia and ethylenediamine salts and their violet colour is destroyed showing the decomposition of the co-ordination group. The specific rotations of the *d*-tartrate, *d*-camphor sulphonate and nitronate were measured.

EXPERIMENTAL.

bis-Propylenediamine-cupric Chlorides.—Two molecules of propylenediamine added to one molecule of cupric chloride dissolved in water gave a pink-violet solution. On evaporation in vacuum a sticky mass was obtained. This was washed with a mixture of alcohol and ether when a solid of deep violet colour was obtained. It was crystallised from water and purified by recrystallisation or in the alternative by dissolving in alcohol and precipitating by means of ether. In the latter case the solid came out paler in colour. On drying in a vacuum desiccator the products obtained in the two cases were found to be identical. The complex chloride is fairly soluble in alcohol but insoluble in ether and acetone. (Found: Cu, 22.81; N, 19.97 [Cupn₂]Cl₂ required Cu, 22.5; N, 19.82 per cent).

bis-Propylenediamine-cupric bromide was prepared by adding two molecular proportions of propylenediamine to aqueous solution of cupric bromide in the cold and evaporating the mixture in vacuum. The solid obtained was at once crystalline and of violet colour. It was purified by recrystallisation from water and afterwards dried in a vacuum desiccator. The complex bromide could also be precipitated from the original solution by alcohol in which solvent it is only sparingly soluble. The solid obtained in this way was pale blue in colour but gave an identical product when dried in vacuum. It is insoluble in ether and acetone. (Found: Cu, 17.45; N, 15.15. $[\text{Cupn}_2]\text{Br}_2$ requires Cu, 17.1; N, 15.07 per cent).

bis-Propylenediamine-cupric iodide was prepared according to the method of Morgan. Cuprous iodide (1 mol.) and propylenediamine (2 mols.) were shaken in water containing a small amount of iodine. An intense violet colour was developed immediately although but little cuprous iodide dissolved. The mixture was heated to 60° while air was bubbled through it. The cuprous salt then passed into solution. The filtered solution was evaporated in vacuum. The sticky mass obtained was washed with a mixture of alcohol and ether and then crystallised from water. The complex iodide could also be precipitated out with alcohol. The compound was analysed after drying in vacuum over sulphuric acid. (Found: Cu, 13.7; N, 12.1. $[\text{Cupn}_2]\text{I}_2$ requires Cu, 13.64; N, 12 per cent). The unstable cupric iodide has thus been stabilised as in the case of the corresponding ethylenediamine salt by Morgan.

bis-Propylenediamine-cupric Sulphate.—Copper sulphate (1 mol.) and propylenediamine (2 mols.) yielded a purple violet solution. Alcohol precipitated the complex sulphate as a solid of sky-blue colour. It was crystallised from a mixture of alcohol and water and dried in a vacuum desiccator. The complex sulphate is insoluble in alcohol, ether and acetone. On dissolving in water, the colour of the solid changed to purple violet. (Found; Cu, 20.7; N, 18.35. $[\text{Cupn}_2]\text{SO}_4$ requires Cu, 20.7; N, 18.2 per cent).

bis-Propylenediamine-cupric Nitrate.—Two molecular proportions of propylenediamine were added to one of copper nitrate in aqueous solution. Alcohol did not precipitate the complex nitrate from the mixture. The solution was evaporated in vacuum and the solid obtained washed with alcohol. The complex nitrate appeared from its solution in water as deep

violet crystals. It is very slightly soluble in alcohol. {Found. Cu, 19.1; N, 25.1. $[\text{Cupn}_2]$ $(\text{NO}_3)_2$ requires Cu, 18.9; N 25 per cent}.

bis-Propylenediamine-cupric Tartrate.—The method of preparation from barium tartrate and *bis*-propylenediamine cupric sulphate was not satisfactory. The action of silver tartrate on the complex chloride was found to be equally unsatisfactory. The best method of preparing the complex tartrate was to add a slight excess of propylenediamine to copper tartrate suspended in water. The solid quickly dissolved giving a deep purple solution from which the compound could be precipitated by methyl alcohol. It was purified by crystallisation from water and finally dried in a vacuum desiccator over sulphuric acid. Alternatively the solution was evaporated almost to dryness on a water bath and the solid which separated pressed upon a porous tile and further purified. (Found: Cu, 17.30; N, 15.58. $[\text{Cupn}_2]$ $\text{C}_4\text{H}_4\text{O}_6$ requires Cu, 17.66; N, 15.57 per cent) Specific rotation obtained with 5% solution at 32° is $+21^\circ$.

bis-Propylenediamine-cupric camphor sulphonate.—Silver salt of camphor sulphuric acid was added to a solution of *bis*-propylenediamine cupric chloride till the precipitate of silver chloride ceased to be formed. The solution separated from silver chloride was deep violet. On evaporation in vacuum, a solid mass was obtained. It was washed with alcohol and crystallised from water. The complex sulphonate appeared as deep violet crystals. It was dried in vacuum. {Found: Cu, 9.1; N, 8.25. $[\text{Cupn}_2]$ - $(\text{C}_{10}\text{H}_{15}\text{SO}_4)_2$ requires Cu, 9.43; N, 8.31 per cent}. Specific rotation obtained with 5% solution at 32° is $+26^\circ$.

bis-Propylenediamine-cupric Hydroxide.—*bis*-Propylenediamine-cupric chloride was dissolved in water and freshly prepared silver oxide was added to the solution. The mixture was stirred thoroughly and after a few hours filtered. The filtered solution was tested free from chloride. The violet coloured filtrate when evaporated in vacuum turned into a dark solid. It was twice crystallised from water and dried in a vacuum desiccator over sulphuric acid. {Found: Cu, 25.74; N, 22.91. $[\text{Cupn}_2]$ $(\text{OH})_2$ requires Cu, 25.87; N, 22.8 per cent}.

bis-Propylenediamine-cupric Nitronate.—*bis*-Propylenediamine-cupric hydroxide was treated with nitrocamphor. Some nitrocamphor dissolved. A little residue left was filtered off. The solution which was of light violet

colour was evaporated in vacuum. The solid obtained was pale pink. It was dried in vacuum. Acetic acid precipitated nitrocamphor from a solution of the complex nitronate. It is insoluble in ether and alcohol. {Found Cu, 10.14; N, 13.76. $[\text{Cupn}_2] (\text{C}_{10}\text{H}_{11}\text{O}_3\text{N})_2$ requires Cu, 10.5; N, 13.9 per cent} Specific rotation obtained with 5% solution at 32° is $+107^\circ$.

bis-Propylenediamine-cupric acetate was obtained from copper acetate (1 mol.) and propylenediamine (2 mol.). The solid mass obtained by evaporation of the mixture of the solutions of above two substances could be obtained in the pure state after repeated crystallisations from water. The complex acetate is insoluble in ether. {Found: Cu, 19.33; N, 16.85; $[\text{Cupn}_2] (\text{CH}_3\text{CO}_2)_2$ requires Cu, 19.27; N, 17 per cent}

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Received July 25, 1939

FATTY ACIDS AND GLYCERIDES OF THE FAT FROM THE SEEDS OF *GARCINIA INDICA* (KOKUM BUTTER).

BY N. L. VIDYARTHI AND C. J. DASA RAO.

Kokum butter, the fat from the seeds of *Garcinia Indica*, has been examined in detail for its component fatty acids and glycerides. By the usual method of lead salt separation and ester fractionation, the component fatty acids have been found to be myristic acid (1.2%), palmitic acid (5.3%), stearic acid (52.0%), and oleic acid (41.5%). The component glycerides of the fat have been determined by resolving the fat into two fractions by systematic crystallisation from acetone. The amount of fully saturated glyceride was determined by successive oxidation of the fat. The component glycerides of the fat in round numbers are tristearin (2%), oleo-distearin (59%), di-oleostearin (21%), oleo-palmito-stearin (14%), oleo-dipalmitin (2%) and palmito-di-olein (2%).

The trees of *Garcinia Indica* (N. O. *Guttiferae*) are found in the Western Coast of Madras and Bombay Presidencies. The seeds contain about 20-25% of solid fat known as "Kokum butter". It is used for edible as well as for some medicinal purpose. The indigenous process for extracting the fat has been to boil the crushed seed with water and the fat skimmed off from the top. The fat has been examined previously by various workers (Hooper, *Agri. Ledger*, India, 1911-12, p. 122; Jamieson, "Vegetable Oils and Fats," p. 76; Bolton, "Oils, Fats and Fatty Foods," 1928, p. 259-61). Heise (*Tropenpflanzer*, 1897, 1, 10) studied the composition of the glycerides by fractional crystallisation of the fat. According to his observations the fat consists mainly of β oleo- $\alpha\alpha'$ -distearin. No other glyceride has been reported in this fat. The glyceride composition of the fat has not been examined by anybody, since then. Heise (*loc. cit.*) examined the fat from the seeds of *Allanblackia Stuhlmanni*, one of the species of the same botanical order and found that this fat as well had oleo-distearin as the chief glyceride. Hilditch and Saletore (*J. Soc. Chem. Ind.*, 1931, 50, 4687) found that this fat consists of about 70% -oleo-distearin, which is the major component glyceride and it also contains 2% of fully saturated glyceride (tristearin) and about 28% of dioleo-monosaturated glycerides. It is probable that the fat from *Garcinia Indica* contains some other glycerides in addition to oleo-distearin.

Recently a number of investigators have adopted different methods for the study of the glyceride composition of various fats (Amberger and Bauch, *Z. Unters. Nahr. Genussm.*, 1924, 48, 371; Hilditch and Lea, *J. Chem. Soc.*, 1927, 3106; Lewkowitch, *J. Soc. Chem. Ind.*, 1933, 52, 2367; Hilditch

and Ichaporia, *ibid.*, 1938, 87, 44) and it is possible now to find out the composition of the glycerides with better accuracy.

In the present communication the component fatty acids and the glycerides of the fat from *Garcinia Indica* are being recorded. The composition of the fatty acids has been determined by the usual method of separation of the solid and liquid acids (Twitchell, *Ind. Eng. Chem.*, 1921, 13, 806) and fractionation of their esters. The composition of the glycerides has been determined by the method recently adopted by Hilditch and co-workers (*J. Soc. Chem. Ind.*, 1938, 87, 44 and subsequent papers) for the fats of fruit-coated seeds.

EXPERIMENTAL.

The fat of *Garcinia Indica* was obtained by extraction of the crushed seeds with carbon tetrachloride. 5 Kg. of the seed gave 1.155 g. of solid fat (23.1% on the seed), a bit dark in colour. The chemical and physical constants of the fat are given in Table I, along with those observed by the other workers.

TABLE I.

	Hooper.	Jamieson	Bolton.	Present workers.
Sp gravity at 40°	0.895	—	—	0.899
*Refrac. Index at 40°	—	1.4565-1.4575	1.4564	1.4571
M p.	41-45°	40-43°	34°	39.40°
Sapon value	186-191	187.191	191.9	189.2
Iodine value (Wijs)	25-34	25-36	36.1	36.7
Free fatty acid in % as oleic acid	—	—	9.7	7.8
Non-saponifiable	—	2.3%	2.3%	1.2%

Composition of the Fatty Acids.—The fat was saponified and liberated fatty acids were resolved into solid and liquid acids by lead salt and alcohol method (Twitchell, *loc. cit.*) The methyl esters of both these fractions were fractionated and the individual acids were identified by their melting points and mixed melting points with authentic samples.

* Calculated from Butyro-refractometer reading

The low boiling fraction of the liquid acid gave an acid (m.p. 53°) when the oxidation product, with alkaline permanganate in the cold (Lapworth and Mottram, *J. Chem. Soc.*, 1925, 1698) was extracted with low boiling petrol. There was no depression in the melting point when mixed with pure myristic acid. Palmitic acid (m.p. 62°) and stearic acid (m.p. 72°) were obtained from the various fractions of the solid acids. The oxidation of the liquid fractions gave dihydroxystearic acid, m.p. 130° either alone or mixed with an authentic sample of Δ^9 10-dihydroxystearic acid.

From these observations and the fractionation data, the component fatty acids of the fat have been calculated as shown in Table II.

TABLE II.

Acids.	Liquid (43%).	Solid (57%).	Total (100).	% On non-sap. free acids.	
				By wt.	Mol %.
Myristic acid	1.15	—	1.15	1.2	1.5
Palmitic acid	1.74	3.5	5.24	5.3	5.8
Stearic acid	—	51.63	51.63	52.0	51.4
Oleic acid	39.28	1.72	41.00	41.5	41.3
Linoleic acid	0.17	—	0.17	Trace	Trace
Non-saponifiables	0.68	0.12	0.8	—	—

Composition of the Glycerides.—The fat was rendered neutral by heating with an excess of sodium carbonate solution and the neutral fat was used for glyceride determination.

Fully Saturated Glyceride.—The neutral fat (102 g.), after repeated oxidation with potassium permanganate in acetone solution (Hilditch and Lea, *J. Chem. Soc.*, 1927, 3106) gave 1.5 g. of a neutral product of fully saturated glyceride having a saponification equivalent of 297.3 and iodine value nil. On crystallisation from ether at 0°, it melted at 69.8°. So this 1.47% of glyceride is mainly tristearin. There might be small quantity of palmito-distearin but the quantity is too small for any further separation or any quantitative estimation.

Examination of the Mixed Glycerides obtained by Crystallisation from Acetone.—The neutral fat (472 g.) was systematically crystallised from dry acetone at about 0°. Two fractions, one least soluble in acetone (A) and the other (B) most soluble were obtained. The component fatty acids of

these fractions were determined by ester fractionations. The results are given in Tables III and IV.

TABLE III.

	A.	B.	Total.
Weight (in g.)	337.8	134.2	472.0
Sapon. equivalent	259.9	294.4	—
Iodine value	29.8	52.5	—
Glyceride by weight %	71.6	28.4	100.0
Molecular %	71.5	28.5	100.0

TABLE IV.

	Composition weight %				Composition mol %	
Palmitic acid	5.7	9.2	6.7	6.3	9.03	7.3
Stearic	60.9	29.5	52.0	60.36	28.80	41.0
Oleic	33.4	61.3	41.3	33.34	61.15	51.7

The palmitic acid contains the small amount of myristic acid and the oleic a trace of linoleic acid.

Both these fractions (A and B) were hydrogenated with nickel catalyst suspended on kieselguhr and the fully hydrogenated products (A—I. V., 1.4 and B—I. V., 2.7) were crystallised from ether, and thus the amount of tristearin of the fully hydrogenated fractions was determined. Tristearin (81.2 mol. %) from A and 83.5 mol. % from B were obtained.

TABLE V.

Fractionation of esters of solid acids at 2 mm. pressure.

Fractions.	B. p.	Wt.	Sapon. equiv.	Iodine value.
S ₁	70-140°	30.0 g.	290.6	3.52
S ₂	140-142°	42	299.7	2.68
S ₃	145-150°	26.5	299.3	2.22
S ₄	150°	17	299.8	2.15
S ₅	150-155°*	9.5	300.0	1.93
S ₆	155°	23.0	299.3	2.00
Residue S	—	18.5	299.6	2.94
		Total	166.5 g	

*Low vacuum,

The glyceride composition of the fat from *Garcinia Indica* calculated from the above observations are given in Table IV.

TABLE VI.

The molecular % of the fatty acids

	A.	B.	Total.
Mol. %	71.5	28.5	100.0
Palmitic acid	4.5	2.8	7.3
Stearic acid	43.5	8.2	51.7
Oleic acid	23.5	17.5	41.0

Component of glycerides (Mol. %)

Tri-C ₁₈ -glyceride	58.0	23.8	81.8
Fully saturated glyceride			
Tri-C ₁₈ (Tristearin)	1.5	—	1.5
Mono-oleo-disaturated glycerides			
Oleo-distearin	56.1	1.8	57.9
Oleo-palmitostearin	13.5	1.3	14.8
Oleo-dipalmitin	—	1.6	1.6
Di-oleo-monosaturated glyceride			
Di-oleo stearin	0.4	21.0	21.4
Palmito-di-olein	—	1.8	1.8
Tri-unsaturated	—	1.0	1.0

There might be a very small amount of tri-unsaturated glyceride but it could not be more than 1% because the molecular % of oleic acid on the total acid is only about 41%.

The following considerations have been applied for the calculation of the data given in Table VI.

Fraction A.—The fully saturated glyceride, tristearin being most insoluble will remain in this fraction along with the oleo-distearin. Most of oleo-palmitostearin, being sparingly soluble in acetone at low temperature will also be found in this fraction.

Fraction B.—This fraction will consist mainly of di-unsaturated glycerides, with a small quantity of di-saturated glycerides. Any tri-unsaturated glyceride being very much soluble in acetone should come to this fraction, but from the component acids no evidence has been found for its presence in this fraction.

In round figures, the fat from Kokum butter contains tristearin (2.0%) oleo-dipalmitin (2.0%), palmito-diolein (2.0%), oleo-distearin (59.0%), dioleo-stearin (21.0%) and about 14.0% of oleo-palmitostearin. Oleo-distearin and dioleo-stearin are the major component of glycerides of this fat.

Borneo tallow and cocoa butter are of special technical interest because the latter is used as a confectionary fat, and the former has often been proposed as a substituent for the latter. The glycerides of these fats are compared here with those of Kokum butter.

TABLE VII.

	Borneo tallow.	Cocoa butter.	Kokum butter.
Oleo-distearin	40	19	59
Oleo-palmitostearin	31	52	14
Stearo-diolein	13	12	21
Palmito-diolein	3	9	2
Oleo-dipalmitin	8	6	2
Fully saturated glyceride	5	2	2
M. p.	33-37°	34-34°	38-39° (Refined fat)

Kokum butter contains very small percentage of palmitic acid (only about 6.7%), whereas cocoa butter has 23.2% and Borneo tallow 18%. Although the percentage of fully saturated glyceride is low, in case of Kokum butter, the high percentage of oleo-distearin tends to make it a harder fat than both Borneo tallow and cocoa butter. Kokum butter, which has not been industrially much utilised, will prove to be a very good fat for confectionary. The refined and deodourised fat has a perfect white colour, and it does not possess any odour and can be compared to a high class hydrogenated fat.

FATTY ACIDS AND GLYCERIDES OF THE OIL FROM SAPOTA SEEDS (*ACHRAS SAPOTA*).

BY N. L. VIDYARTHI AND M. VENKATESH MALLYA.

The seeds of *Acharas sapota* contain about 10% of a liquid fat. The component fatty acids and the glycerides of the oil have been determined. The component fatty acids of the oil are lauric acid (1.6 %), myristic acid (6.2 %), palmitic acid (12.6 %), stearic acid (12.0 %), oleic acid (66.2 %) and linoleic acid (1.4 %). The component glycerides have been calculated to be oleopalmitostearin (5 %), dioleomyrestin (23 %), dioleopalmitin (36 %), dioleostearin (28 %), triolein (5 %) with another 3% of disaturated mono-olein in which probably the saturated acids are lauric, myristic and a little of palmitic acids

Sapota (*Acharas sapota*) belongs to the natural order of sapotaceæ. The evergreen trees of Sapotas are grown extensively in the southern parts of India. It gives a delicious, edible fruit. Each fruit contains two or three seeds, weighing about 0.5 g. The seeds are covered with a hard dark coloured shell, which can be easily removed, if they are fully dried. The seeds are a waste material. They have kernels inside the hard shell, which comprises 50% of the seed and contains 20% of liquid fat. Although the percentage of oil in the seed is low, it may be possible to extract the oil and utilise it for commercial purpose, as the seeds can be obtained very cheap. The present investigation on the physical and chemical constants of the oil, component fatty acids and the component glycerides was taken up with a view firstly to make these data available for commercial utilisation of the oil and secondly to put forward some more evidence towards the general characteristics of the fats from fruit-coated seeds.

EXPERIMENTAL.

The seeds, obtained from the local sapota fruits, were washed, cleaned and dried. 2.5 Kg. of the seeds, crushed to 24 mesh, gave 252 g. of a light coloured oil, when extracted with carbon tetrachloride. The chemical and physical constants are given in Table I.

TABLE I.

Sp. gr. at 31°	0.8725	Acid value (as % oleic acid)	8.94
Refrac. index at 31°	1.463	Reichert-Meissel value (0.5 g. of oil)	2.8
Sapon value	205.4	Hegner value	92.6
Iodine value (Wij's)	59.8	Non-saponifiables	1.8

Component Fatty Acids.

The oil was saponified and the non-saponifiables were extracted from the soap. The mixed acids liberated from the soap were resolved into solid and liquid acids by Twitchel's lead salt and alcohol method (*Ind. Eng. Chem.*, 1921, **13**, 806). Methyl esters from these acids were fractionally distilled at 0.2 mm. pressure as given below.

TABLE II.

	Wt.	%.	Sapon equiv.	Iodine No.
Oil	170 g.	—	273.1	59.8
Mixed acids	154	91.8	274.7	60.99
(S) Solid acids	97.4	64.9	263.8	46.88
(L) Liquid acids	52.7	35.1	283.2	89.76
(S) Methyl ester	276.4	45.46
(L) Methyl ester	296.8	87.34

TABLE IIIA.

Fractionation of esters.

Solid esters (S).				
Fractions.	B. p.	Wt.	Sapon equiv.	Iodine value.
S ₁	105-130°	3.4 g.	235.0	5.9
S ₂	130-132	4.3	242.3	8.7
S ₃	132	4.0	262.5	18.9
S ₄	134	5.8	279.9	27.1
S ₅	135	6.7	285.7	38.6
S ₆	136	15.3	286.3	44.2
S ₇	137-138°	17.5	291.6	58.3
S ₈	138	10.6	297.7	65.1
S ₉	138-140°	11.0	294.1	53.4
Residue	..	8.6	296.4	64.2
Total		87.2		

TABLE IIIb.

Fractions	B. p	Liquid esters (L)		
		Wt	Sapon equiv	Iodine value
L ₁	138-140°	2.8 g.	285.9	85.52
L ₂	142	8.1	295.0	89.93
L ₃	142-145°	5.2	294.8	89.96
L ₄	145	6.7	294.1	94.71
L ₅	155	3.8	294.4	96.03
Residue	—	3.4	294.6	81.77
		30.0		

The individual acids were identified from these fractions by their mixed melting point with authentic samples. The unsaturated acids were identified by their oxidation products with alkaline permanganate in cold (Lapworth and Mottram, *J. Chem. Soc.*, 1925, 1698).

From L₁, lauric acid melting at 43° either alone or mixed pure lauric acid was obtained by petrol ether extraction of the oxidation product. Myristic acid (m.p. 53°), palmitic acid (m.p. 62°) and stearic acid (m.p. 70°) were identified from the different fractions of the solid esters.

Dihydroxystearic acid, melting at 130° either alone or mixed with pure $\Delta^{8:10}$ -dihydroxystearic acid and two tetrahydroxy acids, one melting at 154° (most soluble in hot water) and the other melting at 174° (least soluble in hot water) were identified from the oxidation products of the various liquid fractions.

From these observations the component fatty acids have been calculated as given in Table IV.

TABLE IV.

Acids.	Solid. 64.9 %	Liquid. 35.1 %	Total. % by wt.	Mol. %
Lauric acid	0.4	1.2	1.6	2.2
Myristic acid	2.7	3.5	6.2	7.4
Palmitic acid	10.4	2.2	12.6	13.7
Stearic acid	11.8	0.2	12.0	11.0
Oleic acid	39.6	26.6	66.2	64.2
Linoleic acid	—	1.4	1.4	1.5

The Component Glycerides.

Fully Saturated Glyceride.—Neutral oil (100 g.) (free acids were removed by washing the oil with sodium carbonate solution). was oxidised repeatedly with powdered potassium permanganate in acetone (Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **22**, 447). In the end it gave 0.4 g of a neutral product, which was found to be non-saponifiable. This proves the absence of any fully saturated glyceride.

Separation of the Glycerides by Acetone.—Neutral oil (300 g.) was systematically crystallised from acetone at 0° and 14.5 g of a least soluble fraction were obtained. The component fatty acids of both the fractions, less soluble (A) and more soluble (B) were determined by ester fractionations.

TABLE V.

	(A).	(B).	Total.
Weight (g.)	14.5	285.5	300.0
Sap. equivalent	273.0	275	274.9
Iodine value	29.5	61.4	60.1
% on total oil + non-sap.	4.6	95.4	—
% on glyceride	4.7	95.3	—
Mol. per cent.	4.8	95.2	—

TABLE VI.

	Component acids (weight %.)			Mol % of component acids		
	A. (4.7%)	B. (95.3%)	Mean.	A. (4.8%)	B. (95.2%)	Mean
Myristic acid	Trace	8.1	7.7	—	9.9	9.4
Palmitic acid	33.8	11.5	12.5	34.2	12.7	13.8
Stearic acid	33.0	10.1	11.2	32.8	9.7	10.8
Oleic acid	33.2	68.7	67.1	33.0	65.0	64.5
Linoleic acid	—	1.6	1.5	—	1.6	1.5

Myristic acid recorded here contains a small amount of lauric acid. These component acids agree within experimental limits with those determined directly from the oil.

Tri-C₁₈-glyceride.—Tristearin is not very much soluble in ether at a low temperature. It has been found that it can be quantitatively crystallised from ether at 0°.

In order to find out the quantity of tri-C₁₈-glycerides in this oil, fraction "B" was fully hydrogenated till the iodine value was nearly nil (1·8) and the hydrogenated product was systematically crystallised from ether. It gave 34·2% (mol.) or 34·5% by weight of tristearin, m. p. 71° and equivalent 296·8.

As the fraction "A" was obtained in small quantity it could not be subjected to the similar procedure as B. From the component fatty acids, it has been taken to be oleo-palmitostearin.

The component glycerides of the oil have been calculated from the above observations and given in the Table VII.

TABLE VII.

Molecular % of the acids in fractions A and B.

	A (4·8 %).	B (95·2 %).	Total (100).
Myristic acid	Trace	9·4	9·4
Palmitic acid	1·7	12·1	13·8
Stearic acid	1·6	9·2	10·8
Oleic acid	1·6	62·9	64·5
Linoleic acid	—	1·5	1·5

TABLE VIII.

Estimated % of glycerides in the oil.

	In fraction A (4·8 %)	In fraction B. (95·2 %)	Total (100)
A Fully saturated glycerides	Nil	Nil	Nil
B Tri-C ₁₈ -glycerides	—	32·8	32·8
C. Di-saturated-olein			
Oleo distearin	—	Nil	Nil
Oleo-palmitostearin	4·8	—	4·8
Oleo-di-palmitin	Nil	—	Nil

TABLE VIII (*contd.*)

	In fraction A.	In fraction B	Total
*Oleo myristo-palmitin or oleo-myristolaurin	—	3.0	3.0
Mono-saturated di-olein			
Di-oleo-stearin	—	27.8	27.8
Di-oleopalmitin	—	36.8	36.6
Di-oleomyristin	—	22.8	22.8
Tri-unsaturated glycerides			
Tri-olein	—	5	5

Myristic acid recorded here contains the small quantity of lauric acid oleic acid and a little of linoleic acid

* This 3 % cent. of di saturated-olein could not be oleo-palmitostearin, which will remain in the acetone-insoluble portion. The saturated acids in this glyceride could be only lower acids like myristic, lauric and a little palmitic acid.

In round figures the component glycerides of sapota seed oil may be given as follows : oleo-palmitostearin (5%), di-oleostearin (28%), di-oleopalmitin (36%), di-oleomyristin (23%), tri-olein (about 5%) and about 3% of a di-saturated-mono-olein glycerides in which the saturated acids are myristic, lauric and palmitic acids

The oil contains no fully saturated glycerides. The general glyceride structure of this oil agrees fairly well with the other fats of the *sapotaceæ* seeds. Fully saturated glycerides, which have been found in other *sapotaceæ* fats, are absent in this oil, probably because the percentage of saturated acids is rather low.

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Received August 14, 1939

THE ROLE OF VITAMINS AND CALCIUM IN THE DIET IN THE UTILISATION OF PROTEINS.

BY K. P. BASU AND K. GUPTA.

Absence of vitamin A, B₁, B₂, B-complex or D from the diet has very little effect on the digestibility of casein but increases the biological value. The term B₂ includes all the heat-stable factors of vitamin B complex. Autoclaving decreases the biological value while absence of calcium has no effect.

The determination of the percentage of a digested protein that is retained by an animal or, in other words, of its biological value is always carried out with diets containing adequate amounts of the necessary vitamins and of minerals. In the estimation of the relative nutritive values of proteins from different sources, protein is the only variable factor in the diets. No systematic experiments appear to have been undertaken to find out the role of the different vitamins and minerals in the utilisation of proteins by animals. Some experiments have been carried out to find out the relation between the proportion of proteins in the diet and vitamin B requirement. Thus Funk *et al.* (*Compt. rend Soc Biol.*, 1925, **92**, 997) experimenting with pigeons found that as the proportion of protein in diet increased the vitamin B requirement decreased. On the other hand, Hartwell (*Biochem. J.*, 1925, **19**, 1074) and also Nelson (*J. Home Economics*, 1926, **18**, 383) report that high protein diet increases the need for liberal intake of vitamin B. Finally, Sherman and Gloy (*J. Biol. Chem.*, 1937, **74**, 117) experimenting with rats and with casien as the protein in diets found that varying the percentage of protein in the diet between 12 and 54 had no effect on the period of survival of the rats in the absence of vitamin B. They concluded that in the estimation of vitamin B, basal diets might vary widely in protein content and still yield interchangeable results.

The aim of the present investigation, which was carried out with rats as experimental animals, was to find out the effect of the absence of vitamins A, D, B₁, B₂ and B complex and of calcium on the biological value of a protein (casein) by the balance sheet method. With rats as experimental animals the effect of vitamin C could not be investigated. Except in the determination of the effect of calcium the casein was freed from all vitamins by autoclaving it under pressure and the effect of this treatment on the biological value of casein was first determined. The experiments were all carried out with diets containing 10% protein.

E X P E R I M E N T A L.

The technique of the experiment was the same as that employed in the previous communications from this laboratory (*Ind. J. Med. Res.*, 1938, **26**, 177; 1936, **23**, 789; 1937, **24**, 1001, 1043; *J. Indian Chem. Soc.*, 1939, **16**, 189, 209). In determining the role of vitamins, the casein was freed from vitamins by heating it in an autoclave and the diets contained adequate and constant amounts of salt mixture. The butter-fat was replaced by vitamin-free vegetable fat.

(i) *Effect of Autoclaving*.—All the vitamins and minerals were supplied in equal amounts in both the series. The results are given in Table II.

(ii) *Effect of Vitamin B Complex*.—Equal amounts of vitamins A and D were supplied in the form of cod-liver oil in both the series. Vitamin B complex was supplied in one series in the form of marmite and withheld from the other. The results are indicated in Table III.

(iii) *Effect of Vitamin B₁*.—Vitamins A and D (cod-liver oil) were supplied in both the series. Vitamin B₁ was withheld from one series by previously heating the marmite supplied in an autoclave. The results are indicated in Table IV.

(iv) *Effect of Vitamin B₂*.—Cod-liver oil was supplied in both the series. In one series international vitamin B₁ preparation and autoclaved marmite were daily supplied while autoclaved marmite was not supplied in the other series. Results are to be found in Table V. The term B₂ thus includes all the heat-stable factors of vitamin B complex.

(v) *Effect of Vitamin A*.—Vitamin A was supplied as β -carotene and vitamin D in the form of the international preparation of irradiated ergosterol in one series while β -carotene was withheld from the other. Marmite was given in both the series. Data are given in Table VI.

(vi) *Effect of Vitamin D*.—The experiments were similar to the above except that the international vitamin D preparation was withheld from one series. Results are indicated in Table VII.

(vii) *Effect of Calcium*.—The experiments were performed with unheated calcium-free casein and the vitamins were supplied in both the series. Calcium of the salt mixture as well as calcium carbonate were omitted from one series of rats which did not receive any calcium in their diets. Results are indicated in Table VIII.

Table I represents the nitrogen eliminations with protein-free diets.

TABLE I.

With nitrogen-free ration.

(Figures of intake and excretion represent daily averages)

Rat No	Average wt	Food intake.	Urinary N.	Faecal nitrogen	
				Total.	Per g of food intake
501	225 g.	9.1 g.	54.7 mg	14.34 mg.	1.57 mg.
502	208	8.0	55.95	13.44	1.67
503	210.5	7.1	55.95	11.6	1.63
504	221	9.1	54.07	13.6	1.5
505	222.5	6.5	57.2	14.26	2.19
506	232	9.2	49.9	19.6	2.13
507	224.5	9.9	58.9	11.9	1.2
508	227	9.9	46.1	13.38	1.35
509	219	10	49.6	44.2	2.42
510	222.5	9.25	48.9	21.5	2.32
511	235.5	8.4	45.3	15.1	1.80
512	234	7.8	42.05	12.88	1.65
513	243.5	10	33.6	22.8	2.28
514	241.5	7.5	50.8	15.74	2.1
515	226.5	10	45.6	26.3	2.63
516	236	10	61.9	24.7	2.47
517	214	8.4	44.2	17.2	2.48
518	216.5	7.7	39.0	10.9	1.42

TABLE II.
Effect of autoclaving on the B.V. of casein.

Rat No.	Av wt. body	Diet	Intake		Faecal nitrogen		Food absorbed		Urinary nitrogen		Food utilised	*B V	*D
			Food.	Nitrogen	Total.	Endo	Rxo	Total	Endo	Exo			
513	233.5 g	10% autoclaved casein diet	12.0 g.	191.2 mg	35.48 mg.	27.36 mg	8.12 mg	183.1 mg	86.8 mg	33.6 mg	129.9 mg	70.92	95.8
514	233	10% unheated casein diet	10.42	166.0	32.49	21.89	10.60	155.4	100.8	50.8	105.4	67.83	93.64
515	214	10% unheated casein diet	9.5	151.3	34.83	24.99	9.84	141.5	91.18	45.6	96.92	68.50	93.51
516	226.5	10% autoclaved casein diet	12.56 g	198.0	36.28	31.01	15.27	182.7	143.8	61.9	100.8	55.21	92.25
517	201	10% autoclaved casein diet	10.8	170.3	38.60	26.79	11.81	158.5	118.2	44.2	84.5	53.30	93.1
518	205	10% autoclaved casein diet	11.42	180.1	31.60	16.22	15.38	164.7	108.2	39.0	95.5	57.94	91.5

* In Table II—VIII, B V denotes Biological and D, the digestibility values

TABLE III.
Effect of withholding vitamin B complex (marinite) from the diet.

Rat No.	Av. wt. body	Diet	Intake			Faecal nitrogen			Urinary nitrogen			N excreted.	B. V.	D
			Food.	Nitrogen.	Total	Total	Endo.	Exo	Total	Endo.	Exo.			
511	234 g.	Complete diet.	14.4 g.	196.6 mg.	45.24 mg	25.92 mg	19.32 mg	19.32 mg	117.28 mg.	45.3 mg.	81.43 mg	95.85 mg	54.06	90.17
512	231.5		12.0	163.8	32.73	19.80	12.93	150.87	106.30	42.05	64.25	86.62	57.41	92.11
513	241		10.6	144.7	39.37	24.17	15.20	129.50	72.27	33.6	38.67	90.33	70.14	89.5
514	241		13.2	180.2	39.58	27.72	11.86	168.34	96.36	50.8	45.56	122.78	72.94	93.42
515	223.5	Without marinite.	13.6	185.6	53.54	35.77	17.77	167.83	88.79	45.6	43.19	124.64	74.27	90.43
516	232		14.1	192.5	50.17	34.83	15.34	177.16	116.22	61.9	54.32	122.84	69.34	92.03

(Figures of intake and excretion represent daily averages)

Effect of omitting vitamin B₁ from the diet.

(Figures of intake and excretion represent daily averages).

Rat No.	Av. wt. body	Diet.	Intake			Faecal nitrogen			Food absorbed.			Urinary nitrogen			Food utilized.	B. V.	D.
			Food.	Nitrogen.	Total.	Endo.	Exo.	Total.	Endo.	Exo.	Total.	Endo.	Exo.				
507	210 g.	Complete diet.	10.63 g.	168.4 mg.	22.59 mg.	12.76 mg.	9.83 mg.	148.6 mg.	130.2 mg.	58.9 mg.	71.3 mg.	87.3 mg.	55.05	94.20			
508	218		12.4	166.4	25.25	16.74	8.51	187.9	128.4	46.1	82.3	105.6	56.31	95.65			
509	205	With vit. B ₁ .	10.2	161.5	35.27	24.68	10.59	150.9	104.8	49.6	55.2	95.7	63.40	93.41			
510	204.5		9.46	149.8	33.35	21.95	11.40	138.4	102.7	48.9	53.8	84.6	61.14	92.33			
517	168		9.96	157.8	37.99	24.71	13.28	144.5	101.1	44.2	56.9	87.6	60.62	91.60			
518	202	With vit. B ₁ .	9.25	146.8	24.74	13.13	11.61	134.9	91.1	39.0	52.1	82.8	61.43	92.02			

TABLE V.

Effect of omitting vitamin B₁₂ from the diet.

(Figures of intake and excretion represent daily averages)

Rat No	Av body wt	Diet	Intake		Urinary nitrogen		N ₂ absorbed	Urinary nitrogen		N ₂ deposited	BV	D
			Food	Nitrogen	Total	Endo.	Exo	Total	Endo.			
511	220.8	Complete diet	13.4 g	219.8 mg	34.85 mg.	24.12 mg.	10.73 mg	209.1 mg.	140.4 mg	45.3 mg	95.1 mg	95.20
512	217.5		10.65	174.6	28.57	17.57	11.00	163.6	111.0	42.05	68.95	93.71
513	230		12.40	203.3	42.04	28.26	13.78	189.5	97.8	33.6	64.2	93.24
514	229	With vitamin B ₁₂	9.94	163.0	34.78	20.87	13.91	149.1	99.7	50.8	48.9	91.51
515	210.5	Without B ₁₂	11.32	185.7	38.01	29.78	8.23	177.5	109.4	45.6	63.8	95.62
516	223		13.20	216.5	44.97	32.60	12.37	204.1	135.1	61.9	73.2	94.30

TABLE VI.

Effect of omitting vitamin A from the diet.

(Figures of intake and excretion represent daily averages).

Rat No	Av. body wt.	Diet	Intake		Faecal nitrogen		Food N absorbed		Urinary nitrogen		N deposited	B. V	D
			Food.	Nitrogen.	Total.	Endo.	Exo	Total.	Endo	Exo.			
505	237 g	Complete diet.	11.93 g	142.6 mg.	31.56 mg.	21.75 mg	9.81 mg	132.79 mg	113.84 mg.	57.2 mg.	56.62 mg.	76.17 mg	93.12
506	248.5		9.43	135.4	28.20	20.09	8.11	127.29	107.03	49.9	57.13	70.16	94.11
507	239	Without vitamin A.	9.90	142.05	19.98	11.88	8.10	133.95	101.21	58.9	42.31	91.64	94.30
508	245.5		9.43	135.4	20.13	12.73	7.40	128.00	85.13	46.1	39.03	88.97	94.53
509	232.5		9.91	142.2	30.67	23.98	6.69	135.51	93.52	49.6	43.92	91.59	95.02
510	240		9.92	142.4	35.06	23.01	12.05	130.35	91.22	48.9	42.32	88.03	91.40

TABLE VII.

Effect of omitting vitamin D from the diet.

(Figures of intake and excretion represent daily averages)

Rat No.	Av. body wt.	Diet.	Intake		Faecal nitrogen			Food absorbed.	Urinary nitrogen			Food utilised.	B.V.	D
			Food.	Nitrogen.	Total	Endo.	Exo		Total.	Endo.	Exo.			
501	216 g.	Complete diet	6.7 g.	85.9 mg.	15.55 mg.	10.52 mg.	5.03 mg.	80.97 mg.	90.58 mg	54.7 mg	35.88 mg.	45.09 mg.	55.6	94.15
502	195		9.0	115.7	23.68	15.03	8.65	107.05	102.55	55.95	46.60	60.45	56.47	92.52
503	197	Vitamin D.	7.2	92.6	18.99	11.74	7.25	85.35	86.67 •	55.95	30.72	54.63	64.01	92.17
504	210		9.04	116.2	23.32	13.56	9.76	106.44	93.31	54.07	39.24	67.20	63.13	91.60
511	223.5		7.99	102.8	21.28	14.38	6.90	95.9	78.61	45.30	33.31	62.59	65.27	93.28
512	221.5	Without Vitamin D.	8.35	107.4	19.61	13.78	5.83	101.57	78.91	42.05	36.86	64.71	63.71	94.66

TABLE VIII

Effect of omitting calcium from the diet.

(Figures of intake and excretion represent daily averages)

Rat No	Av body wt	Diet	Intake		Faecal nitrogen			Nitrogen absorbed			Urinary nitrogen			N deposited	B V. D
			Food	Nitrogen	Total	Endo	Exo	Total	Endo	Exo	Total	Endo	Exo.		
501	211 g.	Complete diet	13.9 g.	221.2	36.14 mg	21.82 mg.	14.32 mg.	206.88 mg	114.65 mg.	54.70 mg	59.95 mg.	146.93 mg	71.02	93.52	
502	193		14.0	222.7	38.39	23.38	15.01	207.69	118.05	55.95	62.10	145.59	70.10	93.3	
503	192.5	Without calcium	13.9	221.2	24.87	22.66	2.21	218.99	120.50	55.95	64.55	154.44	70.57	99.00	
504	205		13.9	221.2	22.80	20.85	1.95	219.25	119.19	54.07	65.12	154.13	70.30	99.12	
505	207.5		13.7	218.5	32.30	30.0	2.30	216.20	121.18	57.20	63.98	152.22	70.40	98.95	
506	215		13.3	210.9	30.32	28.33	1.99	208.91	112.32	49.90	62.42	146.49	70.12	99.50	

DISCUSSION

The observed biological value (70.0) of unheated casein at 10% level agrees with the value obtained by previous workers like Mitchell, Boas Fixsen and also by Kik (*Proc. Soc. Exp. Biol. Med.*, 1937, **37**, 129).

Effect of Heat Treatment—There is some controversy regarding the effect of heat treatment on the biological value and digestibility of proteins. Seegers and Schultz (*J. Nutrition*, 1936, **11**, 5, Proc.) observed that autoclaving of beef muscle for 1 hour at 15 lbs. pressure and of casein for 2 hours at 120° or at 150° for 30 minutes had no effect on the biological value of the proteins. Very recently Morgan and Loveen (*J. Nutrition*, 1938, **16**, 115) have observed that heating for 30 minutes at 140° reduced the biological value of casein as tested on rats from 69 to 57—a loss of 17%—and lowered the digestibility by 4%. In this investigation autoclaving the casein at 120° for 2 hours lowered the biological value by about 20% and the digestibility by about 2%. In a previous investigation from this laboratory Basu, Nath and Mukherjee (*Indian J. Med. Res.*, 1937, **24**, 1001) found that heating the soyabean lowered the biological value of its proteins.

Effect of the Absence of the Vitamins—It is remarkable that omitting the vitamins from the diet always increased the biological value of casein in the short term experiments. Of course if the experiments in the absence of vitamins are prolonged for a long time, the rats would lose appetite, take insufficient food and get emaciated. In these short period experiments the rats were not completely depleted of their body store of the particular vitamin although the latter was omitted from the diets. The reason why the absence of a particular vitamin from the diet caused a better retention of the protein was probably that in the absence of the vitamin the metabolism and oxidation of the amino-acids of the protein were depressed which caused a diminished elimination of nitrogen in the urine and hence a higher retention of the nitrogen.

Effect of Calcium.—Recently Ranganathan and Rau (*Nature*, 1938, **142**, 165) reported that the addition of calcium to the Madras diet considerably increased the biological value and digestibility of the proteins of the diet as measured by rat experiments. In the present investigation, which was conducted with calcium-free casein, experiments conducted in the presence of and in the complete absence of calcium from the salt mixture in the diet, gave identical values for the biological value of casein. It appears that calcium has no effect on the biological value of protein.

HALOGENATION. PART XXI. DIRECT REPLACEMENT OF AROMATIC SULPHONIC GROUPS BY CHLORINE AND BROMINE ATOMS

BY PHULDEO SAHAY VARMA, N. B. PAREKH AND V. K. SUBRAMANIAM.

In about 50 aromatic hydrocarbons and their derivatives, the sulphonic group or groups have been replaced by chlorine and bromine atoms by heating strongly the sulphonic acid derivatives or their sodium salts with cupric chloride and cupric bromide respectively.

Sulphonic groups in aromatic compounds are readily replaced by hydroxyl and cyanogen groups by fusing sulphonic acid derivatives with alkali hydroxides and alkali cyanides respectively. No direct replacement of sulphonic groups in aromatic compounds by chlorine or bromine atoms takes place. With a view to replacing the sulphonic group by chlorine atom in benzene sulphonic acid or its sodium salt, the latter has been fused in a glass or copper flask with the chlorides of sodium, potassium, silver, magnesium, lead, zinc, iron (ferric), chromium and copper (both cuprous and cupric).

It is only when sulphonic acid derivatives of aromatic hydrocarbons or of some of their derivatives are heated strongly with copper chloride and bromide (both cupric and cuprous) that sulphonic groups are replaced by chlorine and bromine atoms and chloro and bromo compounds are obtained.

This method has been extended to a number of aromatic sulphonic acid derivatives or their salts or some of their nuclear substituted derivatives, resulting in the formation of the corresponding halogen compounds.

EXPERIMENTAL.

A weighed quantity of the sulphonic acid or its sodium salt was mixed thoroughly with cupric chloride or bromide and the mixture heated strongly over a naked flame for about 2½ hours in a copper distilling flask provided with a condenser and a receiver. In several cases a brisk evolution of sulphur dioxide took place. The products collected in the receiver were ordinarily

The experiments on benzene sulphonic acid alone have been carried with Mr. Subramaniam and the rest of the experiments have been conducted with Mr. Parekh.

coloured, greenish brown in the case of copper chloride and reddish brown in the case of copper bromide. In some cases, the products were obtained as liquids and in others they were partly liquid and partly solid. The products were separated, washed, dried and distilled or crystallised as the case may be and then identified. The yields of the pure halogen compounds obtained from 25 g. of the following sulphonic acids with CuCl_2 or CuBr_2 (quantities varying roughly from 20-25 g.) are summarised below.

Substances used.	Products with yield.
Benzene-sulphonic acid or its Na-salt	... Chlorobenzene (3 g.)
Benzene-sulphonic acid or its Na-salt	.. Bromobenzene (4.25 g.)
Benzene-disulphonic acid	... <i>m</i> -Dibromobenzene (5.1 g.)
<i>o</i> -Bromobenzene-sulphonic acid	.. 1-Bromo-2-chlorobenzene (4.3 g.)
<i>o</i> -Bromobenzene-sulphonic acid	... <i>o</i> -Dibromobenzene (4.3 g.)
2 : 5-Dichlorobenzene-sulphonic acid	... 1 : 2 : 5-Trichlorobenzene (4.1 g.)
2 : 5-Dichlorobenzene-sulphonic acid	... 2 : 5-Dichlorobenzene sulphobromide (1.5 g.) + 1-bromo-2 : 5-dichlorobenzene (1.4 g.)
<i>m</i> -Nitrobenzene-sulphonic acid	... <i>m</i> -Nitrochlorobenzene (4.4 g.)
<i>m</i> -Nitrobenzene-sulphonic acid	... <i>m</i> -Nitrobromobenzene (4.1 g.)
3 : 4-Dichlorobenzene-sulphonic acid	... 1 : 3 : 4-Trichlorobenzene (7.9 g.)
2 : 4-Dichlorobenzene-sulphonic acid	... 1-Bromo-3 : 4-dichlorobenzene (6.8 g.)
<i>p</i> -Toluene-sulphonic acid	.. <i>p</i> -Chlorotoluene (8.1 g.)
<i>p</i> -Toluene-sulphonic acid	... <i>p</i> -Bromotoluene (8.4 g.)
<i>o</i> -Toluene-sulphonic acid	.. <i>o</i> -Chlorotoluene (8.0 g.)
<i>o</i> -Toluene-sulphonic acid	... <i>o</i> -Bromotoluene (8.3 g.)
<i>p</i> -Nitrotoluene-sulphonic acid	... 4-Nitro-2-chlorotoluene (6.5 g.)
<i>p</i> -Nitrotoluene-sulphonic acid	... 4-Nitro-2-bromotoluene (3.5 g.)
<i>o</i> -Xylene-sulphonic acid	... <i>p</i> -Chloro- <i>o</i> -xylene (7.6 g.)
<i>o</i> -Xylene-sulphonic acid	... <i>p</i> -Bromo- <i>o</i> -xylene (7.3 g.)
<i>m</i> -Xylene-sulphonic acid	.. <i>p</i> -Chloro- <i>m</i> -xylene (9.1 g.)
<i>m</i> -Xylene-sulphonic acid	.. <i>p</i> -Bromo- <i>m</i> -xylene (8.1 g.)
<i>p</i> -Xylene-sulphonic acid	... <i>o</i> -Chloro- <i>p</i> -xylene (8.1 g.)
<i>p</i> -Xylene sulphonic acid	... <i>o</i> -Bromo- <i>p</i> -xylene (8 g.)
Mesitylene-sulphonic acid	.. Chloromesitylene (7.2 g.)
Mesitylene-sulphonic acid	... Bromomesitylene (7.2 g.)
Diphenyl- <i>p</i> -sulphonic acid	... <i>p</i> -Chlorodiphenyl (2.5 g.)
Diphenyl- <i>p</i> -sulphonic acid	... <i>p</i> -Bromodiphenyl (2.5 g.)
Naphthalene-1-sulphonic acid	... 1-Chloronaphthalene (7.1 g.)
Naphthalene-1-sulphonic acid	... 1-Bromonaphthalene (4.1 g.)

Substance used	Products with yield
Naphthalene-2-sulphonic acid	2-Chloronaphthalene (1.1 g)
Naphthalene-2-sulphonic acid	2-Bromonaphthalene (1.2 g)
Naphthalene-1, 5-disulphonic acid	1, 5-Dichloronaphthalene (2.4 g)
Naphthalene-1, 5-disulphonic acid	1, 5-Dibromonaphthalene (3.7 g)
Naphthalene-2, 7-disulphonic acid	2, 7-Dichloronaphthalene (3.1 g)
Naphthalene-2, 7-disulphonic acid	2, 7-Dibromonaphthalene (4.6 g)
1-Naphthylamine-4-sulphonic acid	4-Chloro-1-naphthylamine (3.2 g)
1-Naphthylamine-4-sulphonic acid	4-Bromo-1-naphthylamine (3.4 g)
2-Naphthylamine-6, 8-disulphonic acid	6, 8-Dichloro-2-naphthylamine (1.8 g)
2-Naphthylamine-6, 8-disulphonic acid	6, 8-Dibromo-2-naphthylamine (1.1 g)
2-Naphthylamine-4, 8-disulphonic acid	4, 8-Dichloro-2-naphthylamine (2.1 g)
2-Naphthylamine-4, 8-disulphonic acid	4, 8-Dibromo-2-naphthylamine (2.4 g)
<i>o</i> -Toluidine-4, 5-disulphonic acid	4, 5-Dichloro- <i>o</i> -toluidine (1.1 g)
<i>o</i> -Toluidine-4, 5-disulphonic acid	4, 5-Dibromo- <i>o</i> -toluidine (1.2 g)
Phenol- <i>o</i> -sulphonic acid	<i>o</i> -Chlorophenol (2.5 g)
Phenol- <i>o</i> -sulphonic acid	<i>o</i> -Bromophenol (2.6 g)
2-Naphthol-6-sulphonic acid	6-Chloro-2-naphthol (2.8 g)
Cyanobenzene- <i>p</i> -sulphonic acid	<i>p</i> -Chlorobenzonitrile (6.5 g)
Cyanobenzene- <i>p</i> -sulphonic acid	... <i>p</i> -Bromobenzonitrile (6.9 g)
Benzoic acid-sulphonic acid	.. <i>o</i> -Chlorobenzoic acid (7.8 g)
Benzoic acid-sulphonic acid	<i>o</i> -Bromobenzoic acid (7.8 g)

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Received August 10, 1939

NEW ASPECTS OF NITROGEN FIXATION AND CONSERVATION IN THE SOIL PART III INFLUENCE OF LIGHT ON BACTERIAL NUMBERS AND NITROGEN FIXATION.

BY N. R. DHAR AND E. V. SESHACHARYULU.

Nitrogen fixation is always greater in the soils exposed to sunlight than in those kept in dark or covered, while the numbers of azotobacter, total bacteria and fungi are less in the former than in the latter

The optimum temperature for azotobacter is 35° in tropical soils as against 25° (26-30°) in temperate climates. The observed greater nitrogen fixation in the exposed soils is not at all due to the increase of temperature but is caused by sunlight.

On the addition of cellulosic substances, the available nitrogen decreases. Organic manures like cowdung, hay, molasses etc are beneficial because they fix atmospheric nitrogen in the soil

The process of fixation of atmospheric nitrogen in the soil has been believed to be entirely bacterial. Dhar and Mukherji (*Proc. Acad. Sci. U. P.*, 1934, 4, 175) have shown that the fixation of the nitrogen not only takes place in the ordinary soils but also in sterilised soils on the addition of sugars and exposed to sunlight. The authors concluded that nitrogen fixation in tropical soils is not only a bacterial process but is also due to photochemical and induced oxidations going on side by side, since the soil contains oxides of metals like iron, manganese, titanium etc. and is exposed to sunlight for several hours daily.

Further work was felt necessary to decide whether the fixation of nitrogen in the soil is mainly a bacterial process or due also to light and chemical agencies. The present paper deals with the following topics :

1. The nitrogen fixation on the addition of energy-rich substances to the soil.
2. The changes in the numbers of azotobacter, total bacteria. and fungi on the addition of energy-rich substances to the soil.
3. The influence of sunlight on nitrogen fixation, azotobacter, total bacteria and fungi in the soil.

EXPERIMENTAL.

The total nitrogen and carbon were estimated according to the method of Robinson, McLean and Williams (*J. Agric. Sci.*, 1929, 19, 315). The soil

(50 g.) was treated with potassium chloride and magnesium oxide and absorbing the liberated ammonia in dilute sulphuric acid and the ammoniacal nitrogen was determined from the colorimetric estimation of the ammonium sulphate by a Duboscq colorimeter. For nitrate determination Devarda's alloy was used for the reduction of the nitrogen to ammonia.

Methods for Determining the Bacterial Numbers in the Soil.

General procedure.—The soil (0.1 g.) was carefully weighed in a sterile boiling test tube, 10 c.c. of autoclaved tap-water added, the tube shaken well for 5 minutes and then allowed to stand for half a minute. A series of sterile dilution tubes with 9 c.c. of autoclaved tap-water were taken and 1 c.c. of supernatant liquid from the original tube was transferred by a sterile 1 c.c. pipette with proper precautions to the first dilution tube and the mixture well shaken. Further dilutions were carried out in the same way upto a limit which was found out by experience until 1 c.c. of the mixture in the last dilution tube, when plated, allowed a growth of 40 to 300 colonies of bacteria. Platings were done by transferring 1 c.c. from the final and intermediate dilution tubes to sterile petri dishes and pouring 8 to 10 c.c. of the melted media and shaking the petri dishes in the usual way until the media were set to ensure an even distribution of the colonies.

Azotobacter.—Beijerinck's medium sterilised at 14 lbs. pressure for about 15 to 20 minutes was used.

The azotobacter plates were incubated at 30-31° for three days and counted. An average of 5 plates was taken. The types of colonies included in the count were greyish round colonies, round raised concentric, etc.

The total bacteria were determined using Thornton's media with mannitol as energy material and sterilised and counted as above with the difference that the incubation was for 7 days. Here total bacteria include actinomyces and not fungi.

Fungi —The following medium was used :

Glucose	10 g.
Peptone	5.0
KH ₂ PO ₄	1.0
MgSO ₄ , 7H ₂ O	0.5
Distilled water	1000 c.c.

The ingredients were dissolved by boiling and enough normal sulphuric acid added to bring the reaction to pH 3.6-3.8. Agar (25 g. approx.) was

added and dissolved by boiling ; the medium filtered and the final reaction adjusted to p_H 4.8 and sterilised as before.

Sterile containers were used as above without exposing them to air-contamination and the usual procedure was followed. The moisture content of the soils was also simultaneously determined.

Nitrogen Fixation, Azotobacter and total Bacterial counts on the Application of Carbohydrates and Molasses to the Soil in Basins and Fields.

Garden soil (1 kg.) was mixed thoroughly with 20 g. of each of several energy-rich materials and 250 c.c. of water separately in enamelled basins (diameter 26 cm.) and exposed to sunlight for 8 hours daily. Some basins with the same substance were kept in a dark room in order to exclude light. In one set of the experiments with canesugar and glucose 2 g. of calcium carbonate were added. Water (160 c.c.) was added daily to the exposed basins and after every three days to the basins kept in the dark to maintain a uniform moisture content. During summer months 160 c.c. of water were added twice a day to the exposed basins and after every two days to the basins kept in the dark.

In the case of starch and glycerol the experiments were carried out with 5% of these substances. Before commencement of the experiments, estimations of nitrogen, total carbon and azotobacter numbers of the original soils were carried out. At regular intervals the nitrogen content, total carbon and azotobacter numbers of the exposed and dark soils were determined simultaneously. The mean temperature of the soils kept in sunlight and in dark was also recorded. The results are given below.

TABLE I.

Soil (1 kg.) + glucose (20 g.).

A. Exposed to sunlight.

Date.	NH ₃ -N.	NO ₃ -N.	Total N	Total C.	Moisture.	Azotobacter per g. of dry soil in millions.
Original soil						
4-2-36	0.0012%	0.0031%	0.0433%	0.4677%	1.3%	11.2
22-2-36	0.0016	0.0031	0.0433	1.1778	2.1	9.9
7-3-36	0.0028	0.0031	0.0456	1.0014	2.0	17.4
31-3-36	0.0032	0.0032	0.0477	0.8420	1.8	16.3
7-4-36	0.0045	0.0032	0.0500	0.6940	2.1	16.8
21-4-36	0.0038	0.0035	0.0500	0.6420	2.1	16.1
7-5-36	0.0034	0.0038	0.0488	0.5910	2.3	16.2
21-5-36	0.0033	0.0038	0.0488	0.5260	2.1	16.1
7-6-36	0.0030	0.0039	0.0488	0.5180	2.2	16.5

Nitrogen fixed per g. of carbon oxidised = 12.5 mg.

TABLE I (*contd.*):

B. Dark.						
Date.	NH ₃ N.	NO ₃ -N.	Total N.	Total C.	Moiture.	Azotobacter per g. of dry soil in millions.
Original soil						
4-2-36	0.0012%	0.0031%	0.0433%	0.4677%	1.3%	11.2
22-2-36	0.0014	0.0031	0.0433	0.2016	3.0	18.0
7-3-36	0.0016	0.0031	0.0433	1.1224	3.1	37.8
21-3-36	0.0021	0.0031	0.0456	1.0210	3.2	170.0
7-4-36	0.0025	0.0031	0.0466	0.9088	3.6	228.0
21-4-36	0.0028	0.0031	0.0466	0.8078	3.6	320.0
7-5-36	0.0032	0.0032	0.0472	0.6662	3.2	395.0
21-5-36	0.0030	0.0032	0.0472	0.6088	3.3	350.0
7-6-36	0.0029	0.0032	0.0466	0.5546	3.2	345.0

Nitrogen fixed per g. of carbon oxidised = 6.5 mg.

TABLE II.

Soil (1 kg.) + starch (50 g.).

A. Exposed (Temp. 34—48°).

Date.	NH ₃ -N.	NO ₃ -N.	Total N.	Total C.	Moisture.	Azotobacter per g. of dry soil in millions.
Original soil.						
10-3-36	0.0010%	0.0024%	0.0420%	0.4410%	1.6%	7.2
9-4-36	0.0010	0.0024	0.0420	—	2.0	6.8
30-4-36	0.0018	0.0024	0.0433	2.4425	1.9	8.2
20-5-36	0.0023	0.0025	0.0442	2.3700	2.0	9.1
20-6-36	0.0029	0.0025	0.0451	2.2765	2.2	15.6
16-7-36	0.0033	0.0025	0.0461	2.1753	2.5	19.8
28-7-36	0.0037	0.0025	0.0472	2.0932	2.7	28.2
7-10-36	0.0052	0.0026	0.0510	1.4411	3.0	38.6
7-11-36	0.0056	0.0026	0.0530	1.2592	2.8	31.2
7-12-36	0.0058	0.0026	0.0538	1.0181	3.2	39.4
2-1-37	0.0060	0.0026	0.0555	0.7217	3.4	32.8
6-2-37	0.0046	0.0026	0.0560	0.5953	3.5	28.5
2-3-37	0.0040	0.0028	0.0547	0.5684	3.0	16.5
11-4-37	0.0035	0.0033	0.0530	0.5486	3.0	9.5

Nitrogen fixed per g. of carbon oxidised = 7.58 mg.

TABLE II (*contd.*).

B. Dark (Temp. 28—38°).

Date.	NH ₃ -N.	NO ₂ -N.	Total N	Total C.	Moisture.	Azotobacter per g. of dry soil in millions
Original soil.						
10-3-36	0.0010%	0.0024%	0.0420%	0.4410%	1.6%	1.2
9-4-36	0.0010	0.0024	0.0420	—	3.2	8.1
30-4-36	0.0014	0.0024	0.0420	2.5182	3.0	11.2
20-5-36	0.0015	0.0024	0.0420	2.4761	3.5	18.5
20-6-36	0.0018	0.0024	0.0433	2.4137	3.8	26.8
16-7-36	0.0021	0.0024	0.0437	2.3352	4.0	46.0
23-7-36	0.0023	0.0024	0.0442	2.2654	4.2	98.6
7-10-36	0.0031	0.0025	0.0461	1.8286	4.8	205.5
7-11-36	0.0032	0.0025	0.0461	1.7614	4.1	265.5
7-12-36	0.0032	0.0024	0.0466	1.5382	4.6	305.6
2-1-37	0.0035	0.0024	0.0472	1.3124	4.5	365.5
6-2-37	0.0032	0.0025	0.0477	1.0538	3.5	380.0
2-3-37	0.0030	0.0025	0.0482	0.7854	4.0	385.0
11-4-37	0.0024	0.0026	0.0482	0.5368	4.0	300.0

Nitrogen fixed per g. of carbon oxidised = 3.13 mg.

The experimental results show (Table II) that the nitrogen content (both ammoniacal and total nitrogen) and azotobacter numbers increase on the application of carbohydrates to the soils. Exactly similar results have been obtained with other carbohydrates and glycerol. According to the bacterial theory the nitrogen fixation should be parallel to the azotobacter numbers.

But the above results show quite the reverse. The azotobacter numbers in the soils kept in the dark are approximately ten times greater than in the soils exposed to sunlight but the nitrogen fixation is almost double in the exposed soils than in those kept in dark. Moreover, the oxidation of the energy-rich materials is much quicker in the exposed soils than in those kept in dark. Further, the size of the azotobacter colonies developed on the plates containing the soils kept in the dark is much bigger

than those obtained from the exposed ones. A tentative suggestion may be offered as to the reason of the differences in size that the generation time of the azotobacter in the soils kept in the dark is less compared to those in the exposed one probably to sunlight inhibiting the growth. It can be concluded that along with the bacterial fixation of nitrogen in tropical soils, there is considerable fixation due to the photochemical oxidation of the energy-rich substances and that is why although the number of azotobacter in the soils exposed to sunlight is much less than in those kept in the dark the fixation of nitrogen is much higher in sunlight. It also appears that the fixation is not much affected by the addition of calcium carbonate. This behaviour is possibly due to the fact that the soils with which we carried on experiments are rich in calcium. In the case of starch, sugars are detected by Benedict's solution both in the exposed soils as well as in those kept in the dark. Control experiments with blank soil in basins both in light and in darkness were done but no fixation of nitrogen was observed.

Field Experiments.

TABLE III.

Substance.	N fixed per g. of C oxidised.		Substance	N fixed per g. of C oxidised.	
	Sunlight.	Dark.		Sunlight.	Dark.
Canesugar (2%) + CaCO ₃	15.8 mg.	10.5 mg.	Mannitol (2%)	12.8 mg.	6.9 mg.
Canesugar (2%)	14.6	10.2	Dextrin (2%)	13.03	5.98
Glucose (2%) + CaCO ₃	12.5	6.5	Fructose (2%)	11.9	6.8
Glycerol (5%)	6.04	2.76	Maltose (2%)	12.6	6.8
Starch (5%)	7.58	3.13	Galactose (2%)	12.09	6.7

The size of the plots chosen was 4' × 4'. The energy-rich materials were added to the fields (amounts being recorded at the top of each table) with water and thoroughly mixed. The experiments were all carried out under aerobic conditions. Comparative experiments were also carried out by exposing one set of plots to sunlight and another corresponding set with the same amount of substance being covered with wooden planks leaving enough space for aeration but excluding sunlight. The exposed plots were watered after every four days and the covered ones every seven days to maintain a uniform moisture content. Both the covered and the exposed plots were dug up every eight days simultaneously. At regular intervals the nitrogen content, total

carbon, azotobacter and total bacterial numbers were determined. The temperature of the exposed plots during the months of April, May and June varied from 61° to 38° at depths varying from 0 to 6" and that of the covered plots from 41° to 31.5° at the same depths. Control plots were kept, both exposed and covered. In these field experiments after the addition of the energy-rich substances, the soil was immediately taken and the total nitrogen and total carbon were determined.

The mean values of these estimations show that in a plot of area $4' \times 4'$ containing 5 kg. of added glucose and exposed to sunlight the nitrogen fixed per g. of carbon oxidised amounted to 14.0 mg., while in a similar plot, kept covered, the amount of nitrogen fixed per gram of carbon oxidised was 7.26 mg. The effect of adding 4 kg. of starch in a plot of the same area yielded values of nitrogen fixed per g. of carbon oxidised to the extent of 16.5 mg. when exposed to sunlight and 5.9 mg. when kept covered.

These figures show that nitrogen fixation takes place and also azotobacter and total bacterial numbers increase and that the fixation of nitrogen in the exposed plots is always much greater than in the corresponding covered plots, although the azotobacter and total bacterial numbers are less in the former than in the latter. There is no correlation between the nitrogen fixation and azotobacter numbers (*cf.* Waksman, "Principles of Soil Microbiology", 1931, p. 113). The results obtained fully substantiate the conclusion that in tropical soils along with bacterial fixation, photochemical fixation of atmospheric nitrogen is prominent.

Table IV shows the amounts of nitrogen fixed per g. of carbon oxidised both in light and dark or covered.

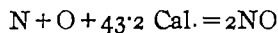
TABLE IV.

Field trials in a plot $4' \times 4'$ containing the carbohydrate.

	Nitrogen fixed per g. of carbon oxidised.	
	Exposed to sunlight.	Dark.
Glucose (5 kg.)	14.0 mg.	7.26 mg.
Molasses (10 kg.)	8.9	3.56
Starch (4 kg.)	16.5	5.9

The above results show conclusively that for the same amount of carbon oxidised the nitrogen fixation in light is much greater than that in the dark. According to Dhar and Mukerji (*Proc. Acad. Sci. U.P.*, 1935, 8, 61)

the formation of nitric oxide may be the first step in the fixation of nitrogen. The formation of nitric oxide according to the equation



is endothermal. A gram molecular weight of these energy materials on oxidation liberates energy much in excess of that required for the combination of nitrogen and oxygen forming nitric oxide. It seems that nitrogen fixation is considerably aided not only by the energy obtained from the oxidation of the carbohydrates but also by the absorption of the incident light.

In the case of starch and glycerol (5%) and molasses (10 kg. in fields) the nitrogen fixed per g. of carbon is small when compared with others because of the high concentrations of the energy materials applied to the soil.

Influence of Temperature and Sunlight on Nitrogen Fixation and Azotobacter Count on the Application of Glucose to the Soil in Basins.

It has been stated (*Nature*, 1937, 139, 894) that the photochemical view of nitrogen fixation has to be tested by carrying on experiments in dark under exactly the same temperature conditions as in sunlight. The following experiments were carried out to examine this aspect.

The garden soil (200 g.) was mixed with pure glucose (4 g.) and moisture content made up to 16% in small enamelled basins (diameter 16 cm.) and incubated at temperatures varying from 11° to 60°. For comparison one basin containing the similarly treated soil with glucose was exposed to sunlight. The results are given below.

TABLE V.

Soil (200 g.) + glucose (4 g.).

A. Exposed to sunlight (mean temp. 42°).				B. Dark (temp 10-12°).		
Date.	Total N.	Total C.	*Azotobacter	Total N.	Total C.	*Azotobacter.
Original soil						
26-2-37	0.0323%	0.3180%	2.05	0.0323	0.3180	2.05
27-2-37	0.0323	1.1168	—	0.0323	1.1156	—
23-3-37	0.0344	0.9512	13.0	0.0323	1.0472	4.0
16-4-37	0.0375	0.7026	20.5	0.0323	0.9186	6.0
13-5-37	0.0411	0.4468	22.5	0.0323	0.7462	4.5
31-5-37	0.0407	0.4356	16.0	0.0323	0.5938	3.5
20-6-37	0.0400	0.4214	14.0	0.0318	0.4684	3.0
10-7-37	0.0396	0.4158	8.5	0.0318	0.3987	2.5

Nitrogen fixed per g of C oxidised = 13.1 mg Nitrogen fixed per g. of C oxidised = nil,

* Expressed as per g. of dry soil in millions.

TABLE VI.

Soil (200 g.) + glucose (4 g.)

Dark (Temp. 35°).

Date.	Total N.	Total C.	Azotobacter per g. of dry soil in millions.
26-2-37	0.0323	0.3180	2.05
27-2-37	0.0323	1.1168	—
23-3-37	0.0338	0.9816	28.0
16-4-37	0.0353	0.7682	110.0
13-5-37	0.0368	0.5618	180.0
31-5-37	0.0375	0.4468	200.0
20-6-37	0.0371	0.4238	160.0
10-7-37	0.0368	0.4199	86.0

Nitrogen fixed per g. of carbon oxidised = 7.76 mg.*

Results of several such experiments at different temperatures together with the nitrogen fixed per g. of carbon oxidised at these temperatures have been recorded below in Table VII.

TABLE VII.

Summary of results obtained at different temperatures.

Temp	Maximum number of azotobacter.*	N fixed.	Temp.	Maximum number of azotobacter.*	N fixed.
42° (Exposed)	22.5	13.1 mg.	40° (Dark)	98.0	3.97 mg.
10-12° (Dark)	6.0	Nil.	45° „	78.0	3.03
25° „	126.0	4.8	50° „	7.5	1.6
30° „	175.0	6.4	60° „	Nil	Nil
35° „	200.0	7.76			

From the foregoing results it is clear that the optimum temperature for nitrogen fixation in the dark is 35° as against about 28° (26°-30°) observed in temperate countries. At 11° and 60° the fixation is nil. The nitrogen fixed in the exposed soil, the temperature of which varied from 40° to 44°, is much greater than that in the incubated soils kept in the dark. The result definitely proves that the increase of temperature in sunlight is not at all the factor responsible for the greater nitrogen fixation observed, which is mainly due to the utilisation of solar energy for nitrogen fixation.

* Expressed as per g. of dry soil in millions.

Another important point that we have observed from the experimental results in basins and fields is that the available nitrogen (the sum of ammoniacal and nitric nitrogen) also increases on the addition of carbohydrates and molasses to the soil. The experience of the other workers in temperate countries is quite different.

In the control plots no increase in the available total nitrogen nor in the bacterial counts is observed.

Nitrogen Fixation, Azotobacter, Total Bacteria and Fungi Counts on the application of Cellulosic substances like Filter paper Cowdung and Hay to the soil in Basins and Fields.

Since doubt has been expressed about the activity of nitrogen fixing organisms in presence of cellulose, the changes in the azotobacter and the total bacterial numbers on the addition of cellulose to the soil were also investigated. Since fungi also take an active part, the change in their numbers was also studied. The following cellulosic substances, *e.g.*, (i) filter paper, (ii) cowdung, (iii) hay were used. Experiments were carried out with cowdung plus hay and hay plus molasses in fields. Analysis of hay and filter paper is shown below

	Hay.	Filter paper.
Total N	0.7778%	
Total C	41.674%	41.56%

The results are given below.

TABLE VII.

Soil (1 kg.) + filter paper (20 g.)

Date.	NH ₃ -N.	A. Exposed.		Total C.	Mois- ture.	Azotobacter per g. of dry soil in millions.
		NO ₃ -N.	Total N.			
Original soil						
30-10-36	0.0011%	0.0020%	0.0540%	0.567%	2.2%	2.4
22-12-36	0.0008	0.0018	0.0540	—	3.8	3.7
20-1-37	0.0007	0.0016	0.0560	—	3.1	7.7
20-3-37	0.0006	0.0014	0.0583	—	3.0	12.5
7-5-37	0.0006	0.0012	0.0646	—	3.5	20.5
7-6-37	0.0006	0.0011	0.0677	—	3.1	27.2
8-7-37	0.0007	0.0014	0.0666	0.7012	—	18.0
13-9-37	0.0014	0.0021	0.0646	0.6704	—	12.0

Nitrogen fixed per g. of carbon oxidised = 18.1 mg (calc.)

TABLE VIII (contd.).

Soil (1 kg.) + filter paper (20 g.).

Date.	NH ₃ -N.	B. Dark. NO ₃ -N.	Total N.	Total C.	Mois- ture.	Azotobacter per g. of dry soil in millions.
Original soil						
30-10-36	0'0011%	0'0020%	0'0540%	0'5670	2'2%	2'4
22-12-36	0'0007	0'0015	0'0540	—	4'8	4'3
20-1-37	0'0006	0'0012	0'0540	—	4'2	5'7
20-3-37	0'0006	0'0010	0'0552	—	4'0	25'5
7-5-37	0'0006	0'0009	0'0567	—	3'0	60'0
7-6-37	0'0006	0'0009	0'0575	—	—	80'0
8-7-37	0'0006	0'0009	0'0583	—	—	92'5
13-9-37	0'0008	0'0001	0'0608	0'6486	—	145'0

Nitrogen fixed per g. of carbon oxidised = 9'2 mg. (calc.).

Identical experiments with 50 g. of cowdung in 1 kg. of soil showed the amount of nitrogen fixed per g. of carbon oxidised to be 20'5 mg. when kept in sunlight as against 6'5 mg. in dark. Similar experiments repeated on a field scale in a plot 4 ft. by 4 ft. gave figures as in tables below.

TABLE IX.

Plot 4 ft. by 4 ft. containing 20 kg. of cowdung.

Date.	Total N	Total C.	A Exposed			
			Moisture.	Azotobacter per g of dry soil in millions	Total bacteria per g of dry soil in millions.	Fungi per g. of dry soil.
Original soil						
10-2-37	0'0323%	0'3294%	1'7%	1'05	10'7	22000
12-2-37	0'0356	0'7126	—	—	—	—
7-3-37	0'0368	0'6324	3'0	6'5	20'5	32000
5-4-37	0'0388	0'5616	3'5	15'8	65'5	41000
29-4-37	0'0424	0'4826	3'0	32'0	160'0	30000
25-5-37	0'0442	0'4108	3'0	30'0	170'0	28000
12-6-37	0'0466	0'3825	3'5	28'5	168'5	28000
28-9-37	0'0446	0'3789	3'2	15'8	115'2	25000

Nitrogen fixed per g. of carbon oxidised = 33'3 mg.

TABLE IX (contd.).

Plot 4 ft. by 4 ft. containing 20 kg. of cowdung.

B. Covered.

	Total N.	Total C.	Moisture	Azotobacter per g. of dry soil in millions	Total bac- teria per g of dry soil in millions.	Fungi per g. of dry soil.
Original soil						
10-2-37	0.0356%	0.3987%	1.8%	1.15	11.2	23000
12-2-37	0.0381	0.7218	—	—	—	—
7-3-37	0.0385	0.6586	4.0	7.5	26.5	40000
5-4-37	0.0392	0.5944	4.0	21.5	112.0	56000
20-4-37	0.0403.	0.5168	3.5	48.5	270.0	89000
25-5-37	0.0411	0.4684	4.0	85.0	315.0	48000
12-6-37	0.0420	0.4158	4.5	65.5	335.0	46000
28-9-37	0.0428	0.4012	5.0	49.5	300.0	41000

Nitrogen fixed per g. of carbon oxidised = 14.6 mg.

TABLE X.

Plot 4 ft. by 4 ft. containing 5 kg. of hay.

A. Exposed.

Date.	Total N.	Total C.	Moisture	Azotobacter per g. of dry soil in millions.	Total bac- teria per g of dry soil in millions	Fungi per gram of dry soil
Original soil						
22-1-37	0.0323%	0.3488%	1.7%	1.5	12.7	27600
3-3-37	0.0350	—	3.0	10.5	25.0	38000
7-4-37	0.0381	—	3.5	20.5	85.6	36000
5-5-37	0.0417	—	3.0	43.5	177.5	33000
29-5-37	0.0442	—	3.5	55.5	220.0	30000
15-6-37	0.0466	—	3.0	45.0	190.0	28000
23-9-37	0.0512	0.6724	4.0	38.5	225.0	29000
28-10-37	0.0500	0.5736	4.5	21.5	195.5	26000

Nitrogen fixed per g. of carbon oxidised = 54 mg.

B. Covered.

Original soil	Total N.	Total C.	Moisture	Azotobacter per g. of dry soil in millions.	Total bac- teria per g of dry soil in millions	Fungi per gram of dry soil
22-1-37	0.0442	0.4199	1.8	1.14	13.3	25100
3-3-37	0.0456	—	4.0	18.0	32.0	43000
7-4-37	0.0472	—	4.5	35.5	140.5	48000
5-5-37	0.0491	—	4.0	55.6	231.0	44000
29-5-37	0.0512	—	4.0	70.0	300.0	46000
15-6-37	0.0525	—	—	82.0	360.0	40000
23-9-37	0.0560	0.7854	5.2	92.8	385.0	41000
28-10-37	0.0560	0.0459	4.8	75.3	315.6	36000

Nitrogen fixed per g. of carbon oxidised = 14 mg.

TABLE XI.

Substance	Nitrogen fixed per g. of carbon oxidised. (Basin experiment).	
	Exposed.	Dark
Cowdung (5%)	20.53 mg.	6.55 mg.
Filter paper (2%)	18.1	9.2
	(Field trials)	
Cowdung (10 kg)	36.58 mg.	16.6 mg.
Cowdung (20 „)	33.33	14.64
Hay (5 „)	54.0	14.0

Greater nitrogen fixation is observed with hay plus molasses and hay plus cowdung than with hay alone. Not much of increase in the numbers of fungi is observed since our soils are not acidic but slightly alkaline (p_H 7.8). The fixation of nitrogen is again much greater and almost double or treble in the exposed soils than in those kept in dark, or covered.

In tropical countries almost a constant yield of crop is possible due to the fixation of atmospheric nitrogen by the oxidation of the energy materials contained in the plant residues left in the soil.

The available nitrogen in the soil decreases in the beginning unlike in the case of carbohydrates, on the addition of cellulosic substances. No increase of total nitrogen and bacterial counts is observed in the control plots without the addition of energy materials.

*Nitrogen Fixation, Azotobacter, Total Bacteria and Fungi Counts on
the Application of Butter and Ghee (clarified butter)
to the Soil.*

According to Rubner (*Arch. Hyg.*, 1922, **91**, 290) only 21.9 % of butter fats added to soil (4.5 g. of fat to 200 g. of soil) were decomposed during a period of one year, and 31.8 % in 12 years; other fats were decomposed at different rates (Waksman, "Principles of Soil Microbiology" 1931, p. 404). The investigation was undertaken to find out whether fats are decomposed in the soil under tropical conditions and any nitrogen fixation takes place as a result of their oxidation. For this purpose butter and ghee (clarified butter) were taken. Since azotobacter is the effective nitrogen-fixing bacteria and a number of aerobic bacteria and fungi take an active part in the decomposition of fats, the changes in the numbers of azotobacter, total bacteria and fungi were carefully investigated. Finally the influence of sunlight on the bacterial counts and nitrogen fixation on the addition of butter and ghee was also studied. The experiments were carried out as in other cases both in basins and in field conditions.

TABLE XII.

Plot 4' x 4' containing 2 kg. of ghee.

A. Exposed.					
	Total N	Total C.	Moisture	Azotobacter per g. of dry soil in millions	Total Fungi per g. of dry soil in millions.
Control (mean)	0.3813%	0.40792%	3.29%	1.454	13.11
Nitrogen fixed per g. of carbon oxidised = 11.7 mg.					

B. Dark					
	Total N	Total C.	Moisture	Azotobacter per g. of dry soil in millions	Total Fungi per g. of dry soil in millions.
Control (mean)	0.0482	0.5213	3.68	1.26	13.93
Nitrogen fixed per g. of carbon oxidised = 4.6 mg.					

Experiments with butter gave similar results.

The foregoing results show clearly that butter and ghee decompose slowly and definite fixation of atmospheric nitrogen takes place as a result of the energy liberated by their oxidation in the soil. It is also evident that when compared to carbohydrates, the oxidation of fats in the soil is a much slower process. The azotobacter and total bacteria increase in number on the addition of butter and ghee to the soil. In this case also the fixation of nitrogen is much greater in the exposed soils than in those kept in dark or covered, whereas the bacterial and fungi numbers are less in the former than in the latter. This clearly indicates that the action of light in the fixation of atmospheric nitrogen when any energy material is added to the soil is a general phenomenon. The available nitrogen, however, decreased in the soil on the application of butter and ghee. In our field experiments, the number of azotobacter in the dark went up to 645 millions per gram of soil on the addition of molasses from 12 millions present in the original un-molassed soil with cellulosic and fatty materials, the increase in the azotobacter numbers in the dark is much less than with carbohydrates, *e.g.*, 93 millions per g. of soil with hay and 94 millions per g. of soil with ghee (clarified butter). In presence of sunlight, however, the increase in azotobacter numbers on the addition of energy materials to soil is always much less than in the dark.

A NOTE ON THE IODINATION OF A FEW HALOGENATED PHENOLS.

BY PHULDEO SAHAY VARMA AND (MISS) K. M. YASHODA.

Phenols and a few substituted phenols can be easily iodinated by means of iodine in potassium iodide solution in presence of concentrated ammonia or nascent nitrogen iodide (Datta and Prosad, *J. Amer. Chem. Soc.*, 1917, **39**, 441). In all these cases poly-iodinated phenols have been obtained. By similar methods *p*-chlorophenol, 2-bromo-*p*-cresol and 4-bromo-*o*-cresol have been iodinated, the first yielding 4-chloro-2-iodophenol (Buchan and Combie, *J. Chem. Soc.*, 1931, 137) and 4-chloro-2 : 6-diiodophenol (Brenans and Girod, *Compt. rend.*, 1928, **186**, 1553 ; Joyce, *J. Amer. Chem. Soc.*, 1917, **39**, 2643), the second yielding 2-bromo-6-iodo-*p*-cresol and the third yielding 4-bromo-6-iodo-*o*-cresol, the last two mixed halogen compounds having been obtained for the first time.

EXPERIMENTAL

4-Chloro-2-iodophenol.—To *p*-chlorophenol (4 g.) dissolved in concentrated ammonia (20 c.c.), a solution of iodine (7.8 g.) in potassium iodide was added slowly with shaking and the products were allowed to stand for $\frac{1}{2}$ hour. After filtration the solution was acidified with dilute hydrochloric acid when a thick oily liquid separated out, which solidified slowly and crystallised in white needles (7 g.) from chloroform, m.p. 78°. It is easily soluble in alcohol and carbon tetrachloride. The *acetyl* derivative, m.p. 57°, is readily soluble in chloroform, carbon tetrachloride and acetone. The benzoyl derivative melts at 83-84° and the picrate melts at 68°.

4-Chloro-2 : 6-diiodophenol.—A concentrated solution of iodine in potassium iodide was added drop by drop with constant shaking to *p*-chlorophenol (4 g.) in concentrated ammonia (20 c.c.) till the colour of iodine persisted. The separated 4-chloro-2 : 6-diiodophenol was crystallised from alcohol in white needles (10.5 g.), m.p. 108°. The *acetyl* derivative melts at 128°. It is also obtained from 4-chloro-2-iodophenol by a similar method.

2-Bromo-6-iodo-p-cresol was obtained from 2-bromo-*p*-cresol (4 g.) and

concentrated ammonia (25 c.c.) in the way described above. It crystallises from alcohol in shining white needles (6 g.), m.p. 46° . It is readily soluble in acetone, alcohol, carbon tetrachloride and chloroform, difficultly soluble in ether. (Found : Total halogen, 65.7. C_7H_6OBrI requires Total halogen, 66.1 per cent). The *benzoyl* derivative melts at 115° .

4-Bromo-6-iodo-o-cresol was obtained from 4-bromo-o-cresol (4 g.) in concentrated ammonia (25 c.c.). It crystallises from chloroform in white needles (5.8 g.), m.p. 49° . (Found : Total halogen, 66.9. C_7H_6OBrI requires Total halogen, 66.1 per cent). The *acetyl* derivative melts at 40° and the *benzoyl* derivative melts at 85° .

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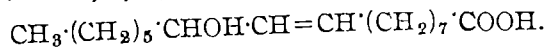
Received August 10, 1939.

A NOTE ON THE OCCURRENCE OF AN ISOMER OF RICINOLEIC ACID IN THE FATTY OIL FROM THE SEEDS OF *VERNONIA ANTHELMINTICA*.

BY N. L. VIDYARTHI AND M. VENKATESH MALLA.

The fatty oil from the seeds of *Vernonia Anthelmintica* (N. O. *Compositae*) has been examined for its physical and chemical constants by Menon (*J. Soc. Chem. Ind.*, 1910, 29, 1431). He does not mention that the oil has got any acetyl value or any optical rotation. We have observed that the oil possesses an optical rotation of $[\alpha]_D^{25}, -10.7$ and an acetyl value of 135.1. The optical rotation and acetyl value are not entirely due to the high percentage of non-saponifiables, generally found in the oil extracted by a solvent. The mixed fatty acids of the oil, free from the non-saponifiables, have been found to have an acetyl value of 118.2 and optical rotation of $[\alpha]_D^{25}, -7.2$. They contain about 60% of the total weight a hydroxy straight chain acid belonging to monohydroxy octadecenoic (ricinoleic acid) series. This acid is sparingly soluble in petroleum ether, which makes its separation from other saturated fatty acids fairly easy. It is viscous just like ricinoleic acid and has a saponification equivalent of 299.0 (calc. for $C_{18}H_{34}O_3$: 298.2).

Bussy and Lecanu (*J. Pharm.*, 13, 57) found that castor oil from the seeds of *Ricinus Communis* contains a high percentage of ricinoleic acid which is *d*-rotatory and has an optical rotation of $[\alpha]_D^{25}, +6.2$ to $+7.5$ (Walden, *Ber.*, 1894, 27, 3472). The constitutional formula assigned to this acid by Goldsobel (*Ber.*, 1894, 27, 3121) and later on verified by Haller and Brochet (*Compt rend.*, 1910, 496) is



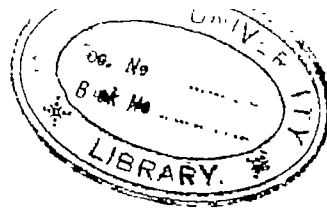
The hydroxy-acid, obtained from the oil of *Vernonia Anthelmintica* seeds differs from ricinoleic acid. It is *l*-rotatory having an optical rotation of $[\alpha]_D^{25}, -7.8$. The hydroxy group appears to be in different position. It has been observed that the hydroxy group is very easily substituted by halogens, even during the period of iodine value determination by Wij's or Hanus solution. When the acid is acetylated and the hydroxy group is protected no more substitution takes place and the exact quantity of iodine only at the ethylenic linkage is absorbed. (I. V. of the acid, 108.4. Found I. V. for acetylated acid 73.8. Calc. for $C_{20}H_{36}O_4$: I. V., 74.4). It differs from the quince oil acid also in that it does not give

a solid dibromide whereas the quince oil acid has been reported to give a dibromide, m.p. 108° (Herrmann, *Arch. Pharm.*, 1899, **237**, 366).

Further work on the constitution of this acid and chemical examination of the other constituents of the oil is in progress.

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Received August 14, 1939.



COMBINED ASCORBIC ACID IN PLANT FOODSTUFFS. PART I.

BY JATINDRA CHANDRA PAL AND B. C. GUHA.

Evidence has been presented to indicate the presence of combined ascorbic acid, "ascorbigen", in several plant tissues. It can be extracted from cabbage by suitable means.

The state in which ascorbic acid is present in certain natural foodstuffs has been the subject of some discussion. Ahmad (*Nature*, 1935, **136**, 797) observed an increase in the ascorbic acid value of cabbage on cooking. A similar increase in cauliflower, carrots, turnips, beets and potatoes on boiling with water was observed by McHenry and Graham (*Nature*, 1935, **135**, 871), who considered that the increase was due to the liberation of ascorbic acid from a combined form, possibly an ester. Van Eekelen (*Nature*, 1935, **136**, 144), working with potatoes, concluded, however, that the observed increase in ascorbic acid value was not due to any such liberation of free ascorbic acid from bound ascorbic acid, but was to be ascribed to the destruction of ascorbic acid oxidase, present in the natural substances, by boiling. Since then Reedman and McHenry (*Biochem. J.*, 1938, **32**, 85) and Scarborough and Stewart (*Biochem. J.*, 1937, **31**, 2232) have presented evidence supporting the presence of combined ascorbic acid in plant tissues and in urine respectively.

The following experiments were planned in order to investigate this problem, preliminary reports of which have been published elsewhere (Guha and Pal, *Nature*, 1936, **137**, 946 ; 1937, **139**, 844). It was considered that solvents like absolute alcohol and ether, which might perhaps extract the combined ascorbic acid from cabbage, would be unlikely to extract the enzyme, ascorbic acid oxidase, and therefore, the behaviour of such extracts to heat should be studied. Secondly, assuming that absolute alcohol would extract some of the oxidase, it was of interest to investigate if exposure of such extracts to a temperature of 36°, which was very unlikely to destroy the enzyme, would cause any increase in ascorbic acid value. Thirdly, the ethereal extract of cabbage, which gave an increased ascorbic acid value on heating, was itself examined and found devoid of ascorbic acid oxidase. Fourthly, it was considered that treatment of the cabbage suspensions and extracts with hydrogen sulphide in the hot and cold conditions should throw light on this question. Whatever dehydroascorbic acid may be formed by the action of the oxidase, all of it will be readily

reduced by hydrogen sulphide in the cold and hence any difference between the results obtained in the hot and cold states is likely to be due to the splitting up of combined ascorbic acid by heat.

The titrations were carried out against the indophenol reagent according to the usual titrimetric method and also in several cases by van Eekelen's procedure or after treatment with formaldehyde in order to eliminate possible interfering substances. All the results indicate that a part of the ascorbic acid present in several foodstuffs investigated is present in the combined form, and further, it has been possible to extract this substance from cabbage in a state practically free from ascorbic acid and attempts are being made to isolate this substance. We have called this combined ascorbic acid, from which ascorbic acid is released on heating, 'ascorbigen' for convenience and brevity.

EXPERIMENTAL.

I. The Ascorbic Acid Values of Cabbage Extracted in Different Ways.

In these experiments titrations were carried out by the usual titrimetric method.

(A) Cabbage was obtained from the local market, cut up as finely as possible from three different cabbages and thoroughly mixed.

(i) 10 G. were taken, ground well in a mortar with sea-sand under 20% trichloroacetic acid and, after addition of water, the mixture was centrifuged. The centrifugate was made up to 100 c.c. and its ascorbic acid content estimated (Table I A, column i).

(ii) Cabbage (10 g.) from the above sample was boiled with water for 5 minutes in air and cooled under the tap; the mixture was extracted with trichloroacetic acid and centrifuged and ascorbic acid was estimated as above (Table I A, column ii).

(iii) The experiment was repeated as under (ii), boiling being carried out in an atmosphere of nitrogen for 5 minutes (Table I A, column iii).

It will be seen from Table I A, which gives results of three different sets of experiments, that the average percentage increases by heating cabbage for 5 minutes in air are 26.5, and for 5 minutes in nitrogen 40.6.

The figures for ascorbic acid would have been higher, if the cabbages, without being cut into pieces previously, had been ground up under trichloroacetic acid, as the oxidase would have been immediately destroyed by the acid. But in our experiments, in order to ensure sampling and comparability of results, the cabbage was first cut into pieces and then divided into different portions to be variously treated. This allowed the

oxidase some time to act on the ascorbic acid. The conditions of experiment were, however, identical and the effect of heat is noticeable.

TABLE IA.

Case	Ascorbic acid per 100 g. of cabbage treated as in		
	(i). Extracted with trichloroacetic acid without any previous treatment.	(ii). Boiled for 5 minutes in air and then extracted.	(iii). Boiled for 5 minutes in nitrogen and then extracted.
1	14.28 mg.	15.38 mg	18.11 mg.
2	12.27	14.28	15.99
3	14.28	22.22	23.53

(B). In this set of experiments van Eekelen's method and formaldehyde treatment were also used for the removal of any possible interfering substances. Sampled cabbage was divided into 10 g. portions. One lot of 10 g. was extracted in the usual way by grinding well with sea-sand in a mortar and under 20% trichloroacetic acid; the centrifugate was made up to 100 c.c. and ascorbic acid was estimated in different portions of it by the following methods.

(i) Ascorbic acid was estimated by titrating against the indophenol reagent (Table I B, column i).

(ii) Ascorbic acid was estimated by titrating against the indophenol reagent after adding 1 c.c. of *M*-formaldehyde solution to the dye (Table I B, column ii).

(iii) The extract was treated first by the mercuric acetate method of van Eekelen, and then the ascorbic acid content was estimated by the indophenol reagent (Table I B, column iii).

Another lot of 10 g. of cabbage from the above sample was heated with water in an atmosphere of nitrogen for 4 minutes, cooled under the tap and ground well with trichloroacetic acid, the ascorbic acid was extracted in the usual way and the volume of the centrifugate made up to 100 c.c. Three different portions of this were estimated by the different methods mentioned above (Table I B, columns iv, v, vi).

Table IB, which gives results of three different sets of experiments, shows that the average percentage increases by heating cabbage for 4 minutes in nitrogen are 201.4 by the ordinary method, 204.1 by the formaldehyde method and 153.9 by van Eekelen's method. This shows strikingly that, whichever the method of estimation employed, there is a remarkable increase in the ascorbic acid value after boiling in nitrogen.

TABLE IB.

(Figures are given in mg. of ascorbic acid per 100 g. of cabbage)

Case	Trichloroacetic acid extract			Boiled in N ₂ for 4 minutes and then extracted with trichloroacetic acid		
	(i). By ordinary titration.	(ii). By adding 1 c.c. M-H CHO to the dye.	(iii). By van Eekelen's method	(iv). By ordinary titration	(v). By adding 1 c.c. M-H CHO to the dye.	(vi). By van Eekelen's method.
1	12.65	12.50	10.30	45.45	45.45	28.87
2	17.42	17.24	15.21	43.47	43.47	34.87
3	15.86	15.86	12.63	47.06	47.06	32.90

(C) Sampled cabbage (10 g.) was ground well in a mortar with sea-sand under trichloroacetic acid and ascorbic acid was extracted by centrifuging with water. The extract was divided into two portions.

(i) One portion was titrated without heating (Table Ic, column i).

(ii) The other portion was heated on a boiling water-bath for 4 minutes in an atmosphere of nitrogen under reflux condenser, cooled under the tap and then the ascorbic acid was estimated (Table Ic, column ii.)

It will be seen from Table Ic that the ascorbic acid content of the trichloroacetic acid extract of cabbage does not increase on heating in nitrogen, which might perhaps indicate that trichloroacetic acid does not extract much of the bound ascorbic acid or that the hot acid has a destructive action on it (*vide* Sec. VIII).

TABLE IC.

(Figures are given in mg. of ascorbic acid per 100 g. of cabbage.)

Case	Trichloroacetic acid extract	Trichloroacetic acid extract heated in N ₂ for 4 minutes
	(i)	(ii)
1	20.83	20.56
2	12.50	8.17
3	17.76	15.90

II. Extraction of Cabbage with Absolute Alcohol.

Sampled cabbage (50 g.) was ground well with anhydrous sodium sulphate (about 200 g. of the sulphate were required). This was taken in a 500 c.c. flask and shaken with absolute alcohol in 2 or 3 instalments (75 c.c. of absolute alcohol each time) and filtered through Buchner funnel. The filtrates were collected and the final volume was determined, to which half its volume of distilled water was added.

Aliquot portions were taken in different conical flasks. One was titrated against the indophenol reagent in the cold, and others were heated at different temperatures and for different periods, some in air and the remaining in an atmosphere of nitrogen, all under reflux condenser, cooled under the tap and were titrated with the indophenol reagent (Table II). The alcoholic extract, which was slightly coloured, was in each case diluted with half its volume of water before titration so as to diminish the intensity of colour.

As will be seen from the table, the average percentage increases are 48.6 by heating the extract on a boiling water-bath for 4 minutes in air, 83.3 by heating similarly for 4 minutes in an atmosphere of nitrogen and 35.0 by heating at 40° for 10 minutes in an atmosphere of nitrogen.

TABLE II.

Case.	Titrated without heating.		Heating in air		Heating in nitrogen		
	Ascorbic acid per 100 g. of substance.	Time of heating.	Condition of heating	Ascorbic acid per 100 g. of substance.	Time of heating.	Condition of heating.	Ascorbic acid per 100 g. of substance.
1	0.48 mg.	4 min.	On boiling water-bath	0.75 mg.	4 min	On boiling water-bath	0.82 mg.
2	0.98	4	Do	1.46	4	Do	1.77
3	0.17	4	36°	0.17	4	36°	0.18
					10	36°	0.19
4	0.72	4	On boiling water-bath	1.20	10	40°	1.17
					4	On boiling water-bath	1.86
5	6.88	4	Do	8.80	10	40°	9.20
					4	On boiling water-bath	9.90
6	6.91	4	Do	9.60	10	40°	7.28
					4	On boiling water-bath	12.55
7	8.92	4	Do	13.80	10	40°	12.36
					4	On boiling water-bath	15.92

III. Extraction of Cabbage with Ether.

Sampled cabbage (50 g.) was ground well with anhydrous sodium sulphate as under Section II, and extracted with ether in 2 or 3 instalments using 75 c.c. of ether each time and filtered through Buchner funnel. The collected filtrate was evaporated by means of a fan. The cream-like residue was taken up in water and the insoluble substance filtered off. The filtrate was made up to a definite volume, one portion of which

was titrated in the cold. Other portions were heated at different temperatures and for different periods in an atmosphere of nitrogen under reflux condenser, cooled and then the ascorbic acid content was determined (Table III).

These results show that free ascorbic acid is not extracted from cabbage by ether under anhydrous conditions and that the ethereal extract, after treatment with water and heating on a boiling water-bath in nitrogen, produces free ascorbic acid invariably, while at 40° increase is observed in several cases. It is apparent that ether extracts some ascorbigen from cabbage.

TABLE III.

Case	Titrated without heating.		Heating in nitrogen	
	Ascorbic acid per 100 g. of substance.	Time of heating.	Condition of heating	Ascorbic acid per 100 g. of substance
1	0 mg.	4 min	On boiling water-bath	0.70 mg.
2	0	10	40°	0.52
		4	On boiling water-bath	0.76
3	0	10	40°	0.48
		4	On boiling water-bath	0.74
4	0	10	40°	No appreciable change
		4	On boiling water-bath	2.45 mg.
5	0	10	40°	No appreciable change
		4	On boiling water-bath	1.29 mg
6	0	30	40°	No appreciable change
		60	40°	Do

IV. Testing of the Ethereal Extract of Cabbage for Ascorbic Acid Oxidase.

A. Sampled cabbage (50 g.) was extracted with ether in presence of anhydrous sodium sulphate as under Section III. The ethereal extract was evaporated by means of a fan, the residue was taken to solution in an acetate buffer of p_H 5.5 (see Tauber, Kleiner and Mishkind, *J. Biol. Chem.*, 1935, **110**, 211), filtered, and the filtrate was made up to a definite volume with the above buffer solution.

B. 2 G. of the above cabbage sample were ground well in a mortar with sand and extracted with buffer of p_H 5.5. The total volume was made up to a definite volume with the buffer.

C. An approximately known weight of pure ascorbic acid was made into a solution with buffer of p_H 5.5 and made up to a definite volume with the buffer.

For testing of the ethereal extract of cabbage for ascorbic acid oxidase, the following experiments were arranged, the total volume in each case being made up to 20 c.c. with buffer of p_H 5.5.

(a) 2 C.c. of ethereal extract (A) + 14 c.c. of ascorbic acid solution (C) + 4 c.c. of buffer.

(b) 2 C.c. of ethereal extract (A) + 18 c.c. of buffer.

(c) 14 C.c. of ascorbic acid solution (C) + 6 c.c. of buffer.

(d) 14 C.c. of ascorbic acid solution (C) + 2 c.c. of aqueous extract (B) + 4 c.c. of buffer.

(e) 2 C.c. of ethereal extract (A) + 18 c.c. of buffer.

(a), (b), (c) and (d) were all taken in similarly corked conical flasks and incubated in a thermostat at 38° for 60 minutes, while (e) was heated on a boiling water-bath under reflux in nitrogen for 4 minutes. The total ascorbic acid contents of all these were estimated and are given in Table IV. The fact that figures under (a) are greater than the sums of the figures under (b) and (c) shows the absence of ascorbic acid oxidase in the ethereal extract and shows, further, that under these conditions also part of the ascorbigen of the ethereal extract splits producing free ascorbic acid. The figures under (d) show the presence of the oxidase in the aqueous extract of cabbage, while figures under (e) show that heating in nitrogen releases a certain amount of ascorbic acid from the ethereal extract, as has been shown in the previous section.

TABLE IV.

Figures are given in mg. ascorbic acid.

Case.	(a).	(b).	(c).	(d).	(e).
1.	7.27	0.010	6.66	—	0.024
2	2.10	0.023	2.00	0.38	0.033
3	0.47	0.007	0.43	0.12	0.024

V. *Extraction of Germinated Mung (Phaseolus Mungo) in Different Ways.*

Kancha mung (5 g.) was kept for germination in a petri-dish with water. The seeds were allowed to germinate for 48 hours. During this period water was added from time to time in small quantities in order to keep them moist. Excess of water was always avoided to prevent putrefaction of the seeds.

A. Aqueous Extract of the Germinated Mung.

The germinated seeds were taken in a mortar, ground well with sea-sand and extracted with water. The total volume of the centrifugate was made up to 100 c.c., which was divided into two portions (a) and (b).

(a) This portion was again divided into the following 3 parts.

(i) Ascorbic acid was estimated in one part by the indophenol reagent (Table VA, column i).

(ii) Ascorbic acid was estimated by the above method, after adding 1 c.c. of *M*-formaldehyde solution to the dye (Table VA, column ii).

(iii) The solution was first treated by the mercuricacetate method of van Eekelen, and then the ascorbic acid content was estimated (Table VA, column iii).

(b) This solution was heated on a boiling water-bath for 4 minutes in an atmosphere of nitrogen under reflux condenser, cooled under the tap and was divided into 3 parts, which were estimated by the different methods, mentioned above (Table VA, columns *iv*, *v* and *vi*).

The results show an increase of the ascorbic acid value on heating in nitrogen, as estimated by all these three methods.

B. Alcoholic Extract of the Germinated Mung.

The germinated *mung* was taken in a mortar, ground well with anhydrous sodium sulphate and extracted with absolute alcohol as under Section II. The alcoholic solution was treated with half its volume of distilled water. One portion of this was titrated in the cold (Table VB, column i). Another portion was heated on a boiling water-bath for 4 minutes in an atmosphere of nitrogen under reflux condenser, cooled under the tap, and then titrated (Table VB, column ii).

C. Ether Extract of Germinated Mung.

The germinated *mung* was ground in a mortar and extracted with ether in presence of anhydrous sodium sulphate as under Section III. The ethereal extract was dried by means of a fan, the residue taken into solution, filtered and the filtrate was made up to a known volume. This was divided into two portions, one portion was titrated in the cold (Table VC, column i) and the other portion was heated on a boiling water-bath for 4 minutes in nitrogen under reflux condenser, cooled and then titrated (Table VC). The values are calculated on the basis of the dry weight of the seeds.

TABLE VA.

Aqueous extract of germinated mung.

Figures are given in mg. ascorbic acid per g. of dry *mung*.

Case.	Cold aqueous extract of germinated <i>mung</i> .			Aqueous extract of germinated <i>mung</i> heated in nitrogen,		
	(i) Ordinary titration.	(ii) Titration in presence of M formaldehyde	(iii) Treatment by van Bekelen's method	(iv) Ordinary titration	(v) Titration in presence of M-formaldehyde.	(vi) Treatment by van Bekelen's method
1	10.00	10.00	7.42	12.50	12.30	9.04
2	12.12	12.12	10.43	13.33	13.33	11.50
3	9.09	9.09	6.40	12.50	12.30	9.30
4	6.45	6.45	4.34	8.88	8.79	6.23
5	6.15	6.10	4.44	8.69	8.69	6.54

TABLE VB

Alcoholic extract of germinated mung.

Case	(i) Ascorbic acid per 100 g. of dry <i>mung</i> before heating in nitrogen.	(ii) Ascorbic acid per 100 g. of dry <i>mung</i> after heating in nitrogen.
1	3.25 mg.	2.90 mg.
2	2.50	2.72
5	2.68	2.93

TABLE VC.

Ethereal extract of germinated mung

Case.	(i) Ascorbic acid per 100 g. of dry <i>mung</i> before heating in nitrogen	(ii) Ascorbic acid per 100 g. of dry <i>mung</i> after heating in nitrogen.
1	0 mg.	1.12 mg
2	0	1.30
3	0	1.41

VI. Extraction of "Bel" (*Aegle Marmelos*) with
Absolute Alcohol and Ether.

A. *Extraction of Bel with Absolute Alcohol.*—50 G. of *bel* sampled from 3 different fruits, were ground well with anhydrous sodium sulphate and extracted with absolute alcohol as under Section II. The alcoholic extract was treated with half its volume of distilled water and divided into aliquots. One portion was titrated in the cold and the others were titrated after heating at different temperatures and for different periods of time in an atmosphere of nitrogen under reflux condenser (Table VIa).

TABLE VIA.

Alcoholic extract of bel.

Case.	Titrated without heating.	Titrated after heating in nitrogen		
	Ascorbic acid per 100 g. of substance.	Time of heating	Condition of heating.	Ascorbic acid per 100 g. of substance
1	9.85 mg.	10 min.	40°	11.50 mg.
		4	On boiling water-bath	12.17
2	7.71	30	40°	9.07
		4	On boiling water-bath	8.07
3	4.30	30	40°	4.60
		4	On boiling water-bath	4.67

B. *Extraction of Bel with Ether.*—50 G. of sampled *bel* were ground well with anhydrous sodium sulphate and extracted with ether as under Section III. The ethereal extract was air-dried, the residue was taken in solution, filtered and the filtrate was made up to a definite volume. The solution was divided into aliquots; one portion was titrated in the cold and the others were heated at different temperatures and for different periods of time in an atmosphere of nitrogen under reflux condenser, cooled under the tap and then titrated (Table VI B).

TABLE VI B.

Ethereal extract of bel,

Case.	Titrated without heating.	Titrated after heating in nitrogen.		
	Ascorbic acid per 100 g. of substance.	Time of heating.	Condition of heating.	Ascorbic acid per 100 g. of substance.
1	0 mg.	60 min.	40°	No appreciable change
		4	On boiling water-bath	0.27 mg.
2	0	4	40°	0.24
		60	40°	No appreciable change

VII. Extraction of Mango with Absolute Alcohol.

Sampled mango (25 g.) was ground well with anhydrous sodium sulphate and extracted with absolute alcohol as under Section II. The volume of the alcoholic extract was measured and diluted with half its volume of distilled water. This was titrated both in the cold and after heating at different

temperatures and for different periods of time under reflux condenser in an atmosphere of nitrogen (Table VII).

Both green and ripe mangoes were used. But in no case was ascorbigen found to be present

TABLE VII.

Case.	Titrated without heating	Titrated after heating in nitrogen.		
		Time of heating.	Condition of heating.	Ascorbic acid per 100 g. of substance.
1 (Ripe)	7.85 mg	60 min. 4	40° On boiling water-bath	6.53 mg 5.68
2 (Green)	18.62	60 4	40° On boiling water-bath	12.67 12.73

VIII. *The Behaviour of the Aqueous Extract of Cabbage to Acids.*

Sampled cabbage (10 g.) was ground well with sea-sand and extracted with water and centrifuged. The clear liquid after centrifuging was diluted to a definite volume. The solution was then kept in six similar conical flasks in aliquot portions.

(a) This was titrated as such.

(b) This was allowed to stand for 1 hour at room temperature (31°) and then ascorbic acid was estimated.

(c) This was made 0.2% acid (equivalent to the hydrochloric acid concentration in gastric juice) with hydrochloric acid, allowed to stand for 1 hour at room temperature (31°), and then ascorbic acid was estimated.

(d) This was heated in an atmosphere of nitrogen on a boiling water-bath for 4 minutes under reflux condenser, cooled and then ascorbic acid was estimated.

(e) This was made 0.2% acid with hydrochloric acid, heated in nitrogen on a boiling water-bath for 4 minutes under reflux, cooled and then ascorbic acid was estimated.

(f) This was made 0.25% acid with trichloroacetic acid, heated in an atmosphere of nitrogen on a boiling water-bath for 4 minutes under reflux, cooled and then ascorbic acid was estimated.

The results are given in Table VIII.

The comparative values under (a), (b) and (c) indicate that 0.2% acid solution (with HCl) causes a considerable splitting up of ascorbigen. The values under (d), (e) and (f) seem to indicate that heating in nitrogen at the normal p_H of the cabbage extract produces a greater increase in

ascorbic acid value than if the heating is carried out in presence of hydrochloric or trichloroacetic acid. Of these two acids again, the former appears to give a larger yield of free ascorbic acid.

TABLE VIII.

Figures are given in mg. of ascorbic acid per 100 g. of cabbage.

Case.	(a).	(b).	(c).	(d).	(e).	(f).
1	2.45	2.02	4.04	9.30	7.54	4.27
2	1.69	1.30	2.70	5.88	4.87	2.79
3	2.33	2.01	3.68	8.77	7.14	4.06

IX. Rate of Splitting of Ascorbigen in the Alcoholic Extract of Cabbage.

Sampled cabbage (50 g.) was ground well with anhydrous sodium sulphate in a mortar and extracted with absolute alcohol as under Section II. The volume of the alcoholic extract was measured and diluted with half its volume of distilled water. The solution was taken in a flask and incubated at 36° in a thermostat. Titrations were made at intervals of 5, 10, 20, 30 and 60 minutes by taking out aliquot portions (20 c.c.) from the flask.

The values are given in Table IX, which show that ascorbigen splits up progressively at 36° throughout 60 minutes.

TABLE IX.

Rate of splitting of ascorbigen in the alcoholic extract of cabbage.

Incubation of 36° for	Ascorbic acid per 100 g. of substance		
	Case 1	Case 2.	Case 3.
0 min.	0.88 mg.	0.89	0.65
5	0.90	0.97	0.68
10	0.95	1.06	0.71
20	1.07	1.20	0.75
30	1.12	1.31	0.80
60	1.22	1.40	0.98

X. Behaviour of Cabbage Suspensions and Extracts to H₂S in the hot and cold Conditions.

(a) Two equal portions (10 g.) of sampled disintegrated cabbage were taken in suspension in water (50 c.c.) Sulphuretted hydrogen was passed for 45 minutes at room temperature (25°) into one portion. The other portion was

treated with H_2S for 30 minutes at room temperature and then the suspension was heated under reflux on a boiling water-bath for 15 minutes while H_2S was being passed. H_2S was removed completely from both the flasks by a current of N_2 and both suspensions were extracted with trichloroacetic acid and titrated in the ordinary way.

(b) Aqueous and alcoholic extracts of cabbage were treated with H_2S as under (a). Results are given in Table X.

TABLE X.

Figures are given in mg. of ascorbic acid per 100 g. of material.

Aq suspensions of cabbage.		Aq extracts of cabbage.		Alcoholic extracts of cabbage.	
Effect of H_2S at 25°.	on boiling water-bath.	Effect of H_2S at 25°.	on boiling water-bath	Effect of H_2S at 25°.	on boiling water-bath
64.5	72.7	40.0	49.5	12.3	20.3
67.0	73.0	34.6	41.6	9.5	15.0
67.8	81.7	38.5	45.2	21.7	35.3
62.5	71.6	40.0	47.2	24.0	30.0
64.0	73.8				

In all cases, it will be seen, there is a difference between the effect of H_2S in the hot and cold conditions. Comparing Table X with Table IA, it will be seen that the values even for the cold H_2S treatment in Table X are much higher than in Table IA, the reason being that in Table IA the oxidase had time to act before extraction, while in Table X whatever dehydroascorbic acid was formed by the oxidase was reduced back to ascorbic acid by H_2S .

DISCUSSION.

The foregoing experiments indicate that part of the total ascorbic acid in such widely different plant foodstuffs as the cabbage, germinated *mung* (*Phaseolus mungo*) and the Indian fruit bel (*Aegle marmelos*) is present in a combined form, from which the free vitamin can be released by heating. The conclusion that this rise in ascorbic acid value on heating cannot be caused solely by the thermal destruction of ascorbic acid oxidase, as has been supposed by van Eekelen, is based on the following considerations: (1) the extract of cabbage with absolute alcohol gives a greatly increased value for ascorbic acid not merely on being heated on a boiling water-bath but also by exposure to a temperature of 36° or 40° for 10 minutes in an atmosphere of nitrogen (Section II). This

procedure is not likely to inactivate the ascorbic acid oxidase, even assuming that the enzyme comes out in the extraction with absolute alcohol.

(2) An extract of cabbage was obtained with dry ether, which showed no dye-reducing power as such but gave an increased value for ascorbic acid on being heated on a boiling water bath in an atmosphere of nitrogen (Section III). Ether would hardly be expected to extract the enzyme.

(3) The ethereal extract of cabbage was directly tested and found to be devoid of the oxidase (Section IV).

(4) Similar results were obtained also when ascorbic acid was estimated by van Eekelen's method or after formaldehyde treatment to eliminate the action of any possible interfering substances (Section Ib).

(5) Similar results were also obtained with alcoholic and ethereal extracts of germinated *mung* and of the Indian fruit *bel* (Sections V and VI).

(6) By heating an alcoholic extract of cabbage at 36° for one hour a progressive increase in ascorbic acid throughout the period was observed (Section IX).

(7) There is a considerable difference in the ascorbic acid values obtained after treatment with hydrogen sulphide in the hot and in the cold conditions. This can hardly be explained on the oxidase theory, as all the dehydroascorbic acid formed would be readily reduced by sulphuretted hydrogen in the cold, and the increased value of ascorbic acid after hot treatment with sulphuretted hydrogen has therefore apparently been produced by heat. This effect is observed not only with suspensions of cabbage in water, where it may be argued that a larger quantity of ascorbic acid has been liberated by greater disintegration of the cabbage substance by heat, but also with aqueous and alcoholic extracts of cabbage, where no such explanation can be valid.

Not only has thus the existence of combined ascorbic acid in these materials been demonstrated, but, as has been shown in Section III, by treatment of cabbage with dry ether it has been possible to extract ascorbigen from cabbage in a state practically free from ascorbic acid. Further studies are proceeding on the nature of ascorbigen.

As has been shown in Section VII, the alcoholic extract of ripe or green mango does not contain any ascorbigen. It would thus seem that not all plant foodstuffs contain ascorbigen.

As shown in Section VIII, in a 0.2% acid solution (with HCl) of cabbage extracts there is a considerable splitting up of ascorbigen in one hour at room temperature. It would seem, therefore, that ascorbigen solutions would undergo similar splitting up by gastric juice in the stomach.

C O N C L U S I O N .

Working with alcoholic and ethereal extracts of cabbage, germinated *kancha mung* (*Phaseolus mungo*) and the Indian fruit *bel* (*Aegle marmelos*), it has been demonstrated that the increase of ascorbic acid value, which these extracts undergo on heating, cannot be due to the destruction of ascorbic acid oxidase but is to be ascribed to the presence of some ascorbic acid in a combined form. It is proposed to call this combined ascorbic acid "ascorbigen".

Ether has been found to be able to extract ascorbigen from cabbage in a state practically free from ascorbic acid. Ascorbigen solutions obtained from cabbage undergo splitting in 0.2 per cent acid solution (with HCl) at room temperature. Ripe and green mangoes have been found to be free from ascorbigen.

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Received September 4, 1939.

COMBINED ASCORBIC ACID IN PLANT FOODSTUFFS. PART II.

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Further evidence has been presented to show the presence of combined ascorbic acid in cabbage. It has been extracted from dried cabbage and biological evidence has been given to show that the substance extracted is combined ascorbic acid.

In the preceding paper (Pal and Guha, *J. Indian Chem. Soc.* 1939, **16**, 481) evidence has been given to show that in several plant tissues part of the ascorbic acid is present in the combined state. In the present work we have attempted to find a solvent which would be most effective for the extraction of ascorbigen from dried cabbage. Chloroform seems to be the best solvent for this purpose, though it does not extract ascorbigen completely (Tables I—IV). It does not extract free ascorbic acid.

The action of hydrogen sulphide on this chloroform extract was investigated both in the hot and cold conditions. It was observed that by cold H_2S treatment no dye-reducing substance was produced, showing that no dehydroascorbic acid was present in the chloroform extract. This should dispose of the point of Mack and Tressler (*J. Biol. Chem.*, 1937, **118**, 740) that the increase in reducing value on heating plant tissues may be due to the production of reducing substances from dehydroascorbic acid. In the present experiments the chloroform extract did not produce any reducing substance by cold treatment with H_2S , showing the absence of dehydroascorbic acid in the extract, while in the hot condition the effects of H_2S and N_2 were similar (Tables V and VI).

Chloroform extracts of ascorbigen can be concentrated considerably simply by extraction with water, which extracts ascorbigen completely while it dissolves only about 20% of the total solids of the chloroform extract.

The nature of the reducing substance or substances formed by heating the chloroform extracts in nitrogen was tested by the action of ascorbic acid oxidase. The reducing substances (64.8–70.6%) disappeared on treatment with the oxidase (prepared from cucumber), which, while it indicated that the bulk of the reducing substances formed by heat was ascorbic acid, also showed the presence of non-specific reducing substances in the combined state in cabbage extracts.

Finally, in order to test whether the chloroform extract of cabbage contained combined ascorbic acid, biological experiments were carried out with these extracts. The curative technique was adopted with guinea-pigs and it was found that the effects of feeding ascorbigen in the form of the chloroform extract and equivalent quantities of ascorbic acid were comparable (Figs. 1–3).

Preliminary results of this work have been published elsewhere (Guha and Sen-Gupta, *Science and Culture*, 1937, 3, 49; Guha and Sen-Gupta, *Nature*, 1938, 141, 974).

EXPERIMENTAL.

Extraction by Different Solvents.

In each of the following experiments, 10 g. of green cabbage were taken, ground with sand and dried completely at 40° in an oven. The ascorbigen of the dried cabbage was extracted with different solvents, the solvents evaporated off by means of an electric fan, the residue treated with water and heated on a water-bath for 10 minutes in an atmosphere of nitrogen and the reducing substances formed were estimated by titrating with 2:6-dichlorophenol-indophenol. All ascorbigen values are given in terms of the ascorbic acid that is produced on heating the ascorbigen preparations in water in an atmosphere of nitrogen. Figures are given in mg. of ascorbic acid per g. of cabbage (Tables I—IV).

TABLE I.

Extraction with ether and benzene.

	1st Expt.	2nd Expt.	3rd Expt.
Free ascorbic acid in green cabbage	0.306 mg.	0.320 mg.	0.312 mg.
Free ascorbic acid in dried cabbage	0.104	0.117	0.113
Ascorbigen extracted with ether (in terms of ascorbic acid)	0.0106	0.0212	0.0213
Ascorbigen extracted with benzene (in terms of ascorbic acid)	0.0127	0.0274	0.0335
Free ascorbic acid in aqueous extract of the evaporated benzene extract	0	0	0
Do ether extract	0	0	0

TABLE II.

Extraction with benzene and acetone.

	1st Expt.	2nd Expt.	3rd Expt.
Free ascorbic acid in green cabbage	0.266 mg.	0.257 mg.	0.265 mg.
Free ascorbic acid in dried cabbage	0.103	0.104	0.104
Ascorbigen extracted with benzene	0.0196	0.0196	0.0193
Ascorbigen extracted with acetone	0.015	0.014	0.015
Free ascorbic acid in the aqueous extract of the evaporated benzene extract	0	0	0
Do acetone extract	0	0	0

TABLE III.

Extraction with benzene and chloroform.

	1st Expt.	2nd Expt.	3rd Expt.
Free ascorbic acid in green cabbage	0.266 mg.	0.285 mg.	0.281 mg.
Free ascorbic acid in dried cabbage	0.114	0.113	0.107
Ascorbigen extracted with benzene	0.0189	0.0179	0.018
Ascorbigen extracted with chloroform	0.0266	0.0241	0.0273
Free ascorbic acid in the aqueous extract of the evaporated benzene extract	0	0	0
Do chloroform extract	0	0	0

TABLE IV.

Extraction with chloroform and petroleum ether (b. p. 40°).

	1st Expt.	2nd Expt.
Free ascorbic acid in green cabbage	0.281 mg.	0.277 mg.
Free ascorbic acid in dried cabbage	0.0912	0.0896
Ascorbigen extracted with chloroform	0.0203	0.0231
Ascorbigen extracted with petroleum ether (b. p. 40°)	0.0179	0.0173
Free ascorbic acid in aqueous extract of the evaporated		
(1) Chloroform extract	0	0
(2) Petroleum ether extract	0	0

Action of Sulphuretted Hydrogen.

(a) In each of the following sets of experiments 15 g. of dried cabbage were taken and ascorbigen extracted with chloroform. After evaporation of the solvent the residue was treated with water and diluted to 50 c.c. This was divided into two equal portions, one of which was heated for 15 minutes in sulphuretted hydrogen and the other in nitrogen.

TABLE V.

Ascorbic acid value after heating in

	H ₂ S.	N ₂ .
1st Expt.	0.293 mg.	0.300 mg.
2nd Expt.	0.431	0.431
3rd Expt.	0.353	0.358

(b) In each of the following sets of experiments, 20 g. of dried cabbage were taken and ascorbigen was extracted with chloroform. The solvent was evaporated off and the residue was treated with water and diluted to 50 c.c. This was divided into four equal portions, which were treated variously (Table VI).

TABLE VI.

	Ordinary titration without heating	After heating in N ₂ •	After heating in H ₂ S.	After treatment with H ₂ S in the cold.
1st Expt.	0 mg.	0.0542 mg.	0.0542 mg.	0 mg.
2nd Expt	0	0.071	0.071	0

Concentration of Ascorbigen.

The dried cabbage (15 g.) was treated with chloroform at 40° for 2 hours with intermittent shaking. The chloroform extract was taken in a weighed glass basin and evaporated and kept in a vacuum desiccator overnight and then the basin with its contents reweighed. In two experiments, the weights of the residues were 0.1782 g. and 0.2244 g. respectively. On extracting these residues with 15 c.c. of water, only 0.0300 g. and 0.0304 g. respectively came out in the aqueous solution, i.e., only about 20% and 12% respectively of the chloroform-soluble solids were extracted with water and the aqueous fractions contained practically all the ascorbigen which amounted to 0.13 mg. in terms of ascorbic acid in each case.

Action of Ascorbic Acid Oxidase on Concentrated Chloroform Extracts.

The chloroform extract of 10 g. of dried cabbage was evaporated and treated with water. This was centrifuged and the centrifugate diluted to 30 c.c. after heating in an atmosphere of N₂.

TABLE VII.

	A s c o r b i c A c i d				
	1st. Expt.	2nd Expt.	3rd. Expt	4th Expt	5th. Expt.
(1) Ordinary titration (10 c.c.)	0.0346 mg.	0.0416 mg.	0.0378 mg.	0.0346 mg	0.0378 mg.
(2) 10 C.c. of this solution + 3 c.c. of acetate buffer (p _H 5.6) + 2 c.c. water at 40° for ½ hour.	0.0346	0.0416	0.0378	0.0346	0.0378
(3) 10 c.c. of solution + 3 c.c. of acetate buffer (p _H 5.6) + 2 c.c. oxidase solution at 40° for ½ hour.	0.011	0.0118	0.0120	0.0123	0.0132
(4) Percentage of oxidation of the heat-produced reducing substances by oxidase.	68.5	70.6	66.0	64.8	65.0

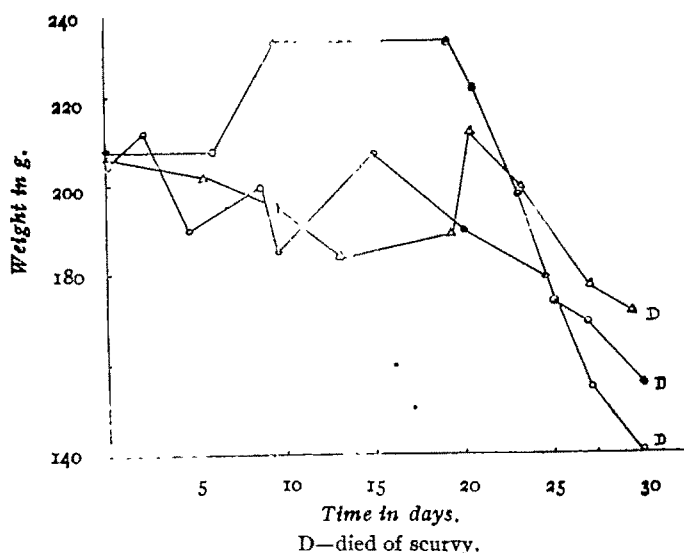
Control experiments were carried out with (1) 10 c.c. of ascorbic acid solution containing the same amount of ascorbic acid as was obtained in the above experiments + 3 c.c. of acetate buffer (p_H 5.6) + 2 c.c. of oxidase solution at 40° for $\frac{1}{2}$ hour and (2) the same mixture in which 10 c.c. of ascorbic acid solution were replaced by 10 c.c. of water. No values for ascorbic acid were obtained in these experiments.

Biological Experiments with Ascorbigen.

Biological experiments were carried out with male guinea-pigs weighing between 190 and 250 g. Their diet consisted of powdered gram (20 parts), powdered oats (80 parts), and salt mixture (1 part) and each animal was given daily 25 c.c. of milk, autoclaved for $\frac{1}{2}$ hour at 10 lb. pressure. The animals were divided into 5 groups. The first group was given scorbutic diet alone. They developed typical signs of scurvy and died (Fig. 1). The second group of animals received 2 mg. of synthetic *l*-ascorbic

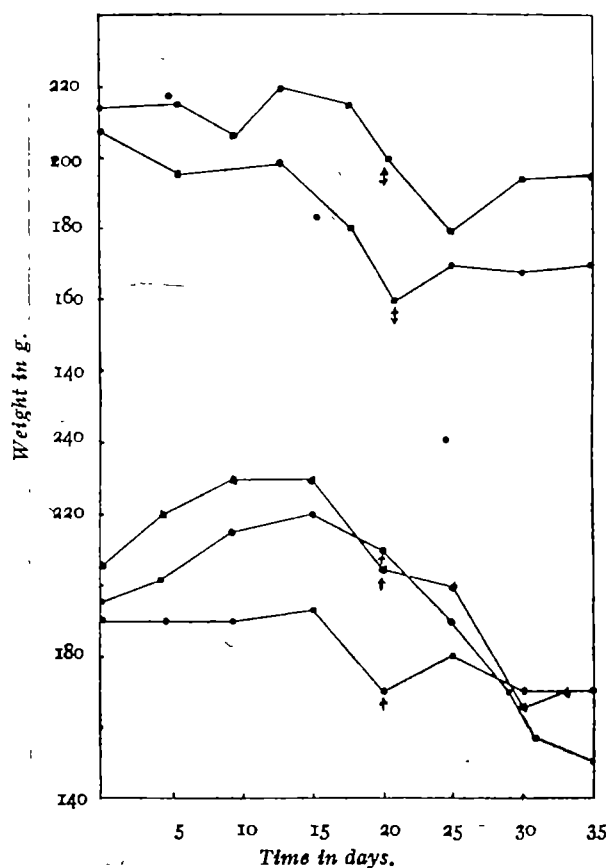
FIG. 1.

Animals on a scorbutic diet.



acid daily as a supplement to the scorbutic diet after their weight began to fall (Fig 2). The third group of animals had a similar supplement with 2 mg. of an ascorbigen preparation (chloroform extract) equivalent in reducing substances as measured by titration with the indophenol indicator to 2 mg. of ascorbic acid. As true ascorbic acid was in fact only about 60-70%

FIG. 2.



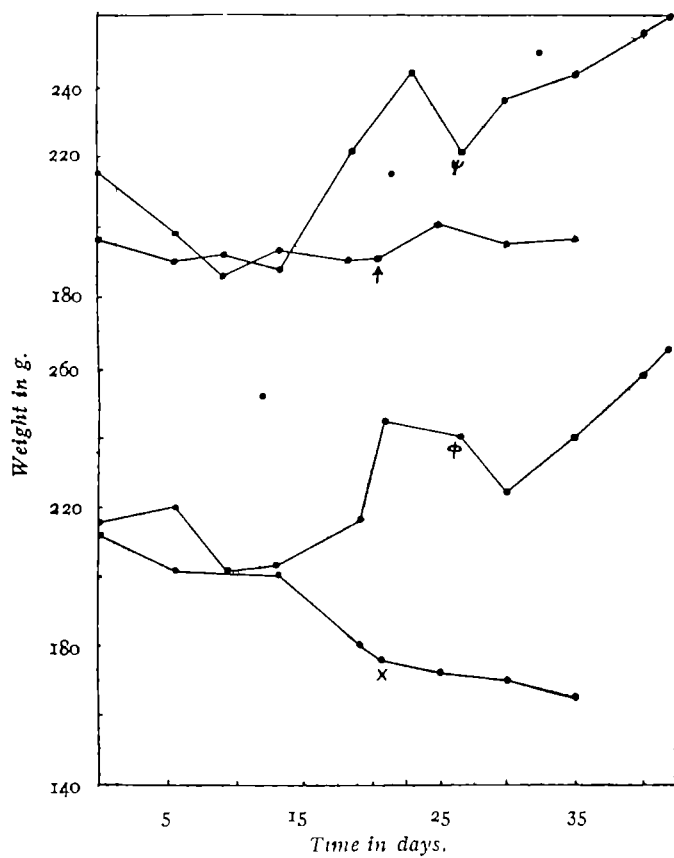
Scorbutic diet supplemented with

↑ ascorbigen preparation equiv. to 2 mg. of reducing substance,

‡ 2 mg. of ascorbic acid.

of the total reducing substances, this dose gave slightly less than 2 mg. of ascorbic acid equivalent. The curves for these animals are given in Fig. 2. for comparison. The fourth group of animals received 4 mg. or 5 mg. of synthetic *L*-ascorbic acid and the fifth group received equivalent amounts of reducing substances present in the ascorbigen preparation obtained from dried cabbage (Fig. 3). It will be observed that the curves for groups of animals on comparable doses of ascorbigen and ascorbic acid are comparable, although those animals which were receiving ascorbigen were actually receiving slightly less ascorbic acid than the animals on pure ascorbic acid supplement.

FIG. 3.



Scorbutic diet supplemented with

φ ascorbigen preparation equiv. to 5 mg. of reducing substance

ψ — " " " " 4 " " "

ψ — 5 mg. of ascorbic acid.

↑ — 4 mg of " "

DISCUSSION

It has been shown that chloroform can extract ascorbigen from dried cabbage to a certain extent. This extract does not decolorise the indophenol

indicator, showing the absence of free ascorbic acid. But when heated in nitrogen it gives a dye-reducing value, about 60-70% of which disappears on treatment with ascorbic acid oxidase. This shows that the bulk of the reducing substances is probably ascorbic acid. While, therefore, there is ascorbigen present in the chloroform extracts, these experiments indicate the presence of other non-specific reducing substances in a combined state.

The reducing substances formed by heating the chloroform extract cannot be degradation products of dehydroascorbic acid as suggested by Mack and Tressler (*loc. cit.*), as the chloroform extract does not give any dye-reducing value on treatment with sulphuretted hydrogen in the cold and therefore does not contain any dehydroascorbic acid.

Biological experiments show that the chloroform extracts are active and their activity is comparable with that of roughly equivalent quantities of pure ascorbic acid. It should be noted that in these biological experiments the ascorbigen preparation contained about 30% less ascorbic acid than the reduction value would indicate, as about 30% consists of non-specific reducing substances. The performance of the animals on ascorbigen was nevertheless comparable with that of animals on pure ascorbic acid.

It has been shown that considerable concentration of ascorbigen can be effected by evaporating the solvent from the chloroform extract of cabbage and then extracting with water. This process extracts all the ascorbigen of the chloroform extract but only about 20% of the total solids. Chloroform, however, does not extract ascorbigen completely from dried cabbage and in subsequent work we have adopted other methods for concentration of ascorbigen.

CONCLUSION.

Chloroform has been found to be the most effective solvent for the extraction of ascorbigen from dried cabbage. The chloroform extract does not reduce the indophenol indicator in the cold and is, therefore, free from ascorbic acid. Treatment with sulphuretted hydrogen in the cold does not produce any dye-reducing value, showing the absence of dehydroascorbic acid, but when heated in an atmosphere of hydrogen sulphide or nitrogen, dye-reducing substances are produced in equal quantities.

About 60-70% of the reducing substances produced by heat disappear on ascorbic acid oxidase treatment, from which it appears that, apart

from ascorbigen, cabbage contains some non-specific reducing substances in a combined state. Biological tests with guinea-pigs by the curative method, in which equivalent quantities of an ascorbigen preparation (chloroform extract of cabbage) and pure ascorbic acid were fed, give comparable results.

By extracting dried cabbage with chloroform, evaporating the solvent and extracting with water, some concentration of the ascorbigen can be effected.

Our thanks are due to the Indian Research Fund Association for financing the researches on ascorbic acid.

DEPARTMENT OF APPLIED CHEMISTRY,
UNIVERSITY COLLEGE OF SCIENCE,
CALCUTTA.

Received September 4, 1939.

CONCENTRATION OF ASCORBIGEN FROM CABBAGE.

BY BAIDYANATH GHOSH AND B. C. GUHA.

Ascorbigen (combined ascorbic acid) has been concentrated from cabbage juice by adsorption with "active" charcoal and by elution with a chloroform-alcohol mixture. Some of the properties of the ascorbigen concentrate are described.

In preceding papers (Pal and Guha, *J. Indian Chem. Soc.*, 1939, **16**, 481; Sen-Gupta and Guha, *ibid.*, p 496) cabbage has been shown to contain ascorbigen (or combined ascorbic acid) by both chemical and biological methods. It was shown further that chloroform extracts of dried cabbage contained ascorbigen and some other non-specific reducing substances in a combined state but no free ascorbic acid. Although evaporating off the solvent from the chloroform extract and taking up the residue in water followed by centrifugation produced a considerable concentration of the ascorbigen of the chloroform extract, the yield of ascorbigen so far as the fresh cabbage was concerned was not satisfactory, as chloroform did not extract all the ascorbigen of cabbage (Sen-Gupta and Guha, *loc. cit.*)

In subsequent experiments we have started with cabbage juice. Glacial metaphosphoric acid produced a precipitate, but it was found that by this means only a fraction of ascorbigen could be precipitated along with some proteins and the main bulk of ascorbigen remained in the filtrate. Similar observations have been made by Scarborough and Stewart (*Nature*, 1938, **142**, 40). In order to precipitate ascorbigen completely from the cabbage juice different protein precipitating reagents were employed without success.

The next attempt was to concentrate ascorbigen from the cabbage juice by suitable adsorbing materials. Fuller's earth, charcoal ("Norit") and active charcoal (Schering-Kahlbaum, "carbo-active") were employed and it was found that charcoal could adsorb the largest amount of ascorbigen from the cabbage juice. The concentration of ascorbigen by charcoal adsorption and subsequent elution by suitable solvents is described in this paper. Further work on the nature of ascorbigen so eluted and its further purification is in progress.

EXPERIMENTAL.

Separation of Ascorbigen from Cabbage Juice by Glacial Metaphosphoric Acid.

Fresh cabbage (1 kg.) was minced in a hand mincer and cabbage juice was pressed out by pressing in a screw-press. The juice obtained measured

500 c.c. Water (100 c.c.) was then added to the pressed cabbage pulp and a further yield of the cabbage juice was thus obtained. The total volume of the juice expressed measured 600 c.c. This was centrifuged in order to remove insoluble matter. Glacial metaphosphoric acid was added to the clear juice so as to make the acid concentration 5%. The precipitate obtained after adding glacial metaphosphoric acid weighed 9 g. (moist). About 0.5 g. of this precipitate was suspended in 15 c.c. of distilled water. 10 c.c. of this suspension were centrifuged and the clear liquid obtained titrated with 2,6-dichlorophenol-indophenol (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935, 12, 30) in order to estimate the free reducing substance present. No reduction of the dye was observed. The remaining 5 c.c. of the suspension were made up to 10 c.c. by the addition of distilled water and this suspension was heated on a water-bath in an atmosphere of carbon dioxide for 10 minutes. The suspension was then cooled under water while carbon dioxide was allowed to bubble. It was then centrifuged and the clear liquid after making up to 10 c.c. was titrated with the dye as usual. The amount of reducing substance liberated by heat treatment, when calculated as ascorbic acid, was found to be 0.312 mg. of ascorbic acid per 0.5 g. of the metaphosphoric acid precipitate. Thus the total 9 g. precipitate contained about 5.6 mg. of ascorbic acid in a combined state.

The filtrate remaining after glacial metaphosphoric acid precipitation when similarly heated for 10 minutes in an atmosphere of carbon dioxide released a considerable amount of reducing substances. The amount of ascorbigen remaining in the centrifugate when expressed in terms of ascorbic acid was found to be 29 mg., showing that the larger proportion of ascorbigen was not precipitated by metaphosphoric acid under our conditions of experiment.

Different Protein-precipitating Reagents and Ascorbigen Concentration.

Since a considerable amount of ascorbigen remained in the filtrate after metaphosphoric acid precipitation it was considered desirable to study the behaviour of different protein-precipitating reagents like trichloroacetic acid, ammonium sulphate, alcohol, etc., with regard to the complete precipitation of ascorbigen from pressed cabbage juice. Different aliquots of juice were taken and different protein precipitating reagents employed. A portion of the filtrate after the separation of the protein was directly titrated with the dye while another portion was heated in an atmosphere of carbon dioxide on a boiling water-bath for 10 minutes. The difference

between these two values gave the amount of reducing substance expressed in terms of ascorbic acid present in the filtrate as ascorbigen. The results are given in Table I.

TABLE I.

Figures are given for 100 c.c. of cabbage juice.

No of expt.	Reagents employed (giving final concentrations).	Ascorbic acid in the filtrate		Ascorbigen present in the filtrate in terms of ascorbic acid.
		Initially present	Obtained after heat treatment	
1	Juice alone	0.57 mg	4.3 mg.	3.73 mg.
2	5% CCl_3COOH	0.57	3.7	3.13
3	<i>m</i> - HPO_3 (5%)	0.67	4.3	3.65
4	$(\text{NH}_4)_2\text{SO}_4$ (40%)	0.43	4.3	3.87
5	Alcohol (50%)	0.52	3.4	2.88
6	HCl (5%)	0.52	4.0	3.48

It was thus observed that in all cases most of the ascorbigen remained in the filtrate and the method of precipitating ascorbigen by these protein-precipitating reagents was, therefore, discarded. Lead acetate and basic lead acetate were also tried but were found largely ineffective in precipitating ascorbigen.

Adsorption Methods.

Since the main bulk of ascorbigen remained in the solution after treatment with protein-precipitating reagents, a suitable method of adsorbing ascorbigen was sought. Among the different materials employed, fuller's earth, "norite" charcoal and "active" charcoal (Schering-Kahlbaum, "carbo-active"), the last was found to be the best, norite charcoal almost equally good and fuller's earth (at p_H 6.2) was ineffective. It was found that by charcoal adsorption about 60% of ascorbigen in the cabbage juice could be adsorbed. When cabbage juice was shaken in a mechanical shaker for 1-1½ hours with charcoal (1.15 g. per 100 c.c. of cabbage juice) the ascorbigen was adsorbed on the charcoal. The amount of ascorbigen adsorbed by charcoal could be estimated by the difference in dye value obtained before and after charcoal adsorption by the usual method of heat treatment in

carbon dioxide. In order to obtain maximum adsorption, adsorption at different p_H values was tried and the results are tabulated in Table II. The choice of variation of p_H is limited as both at low and high p_H values ascorbigen is unstable.

TABLE II.

No. of expt.	p_H	Amount of ascorbigen in terms of ascorbic acid present per 100 c.c. of cabbage juice	
		Before charcoal adsorption.	After charcoal adsorption.
1	6.2	2.7 mg.	0.94 mg.
2	6.4	2.7	0.96
3	6.8	2.3	0.86
4	5.0	1.8	0.90
5	4.0	1.8	0.65

Elution of Ascorbigen from the Charcoal Adsorbate.

The next problem was to elute the ascorbigen from the charcoal adsorbate without the ascorbigen being split up. It was found that ascorbigen could be eluted practically in tact but not quantitatively if the pressed moist charcoal adsorbate was heated under reflux with a mixture of 30% chloroform and 70% absolute alcohol. For every 10 g. of charcoal used 100 c.c. of the chloroform-alcohol mixture were employed. The whole charcoal adsorbate was taken in a round-bottomed flask, chloroform-alcohol mixture was added and the mixture heated under reflux on a water-bath at a temperature of 70-80° for 1 hour, after which the charcoal was filtered under suction. The filtrate, contained ascorbigen, which could be concentrated under reduced pressure at ordinary temperature (30°). As soon as chloroform was evaporated a precipitate was obtained which contained very little ascorbigen and was, therefore, discarded after centrifuging. The remaining alcoholic extract was evaporated to dryness in a vacuum desiccator. The whole chloroform-alcohol extract may also be directly evaporated over calcium chloride in a vacuum desiccator. A second extraction of the charcoal adsorbate gave a further yield of ascorbigen.

Further Purification and Properties of the Ascorbigen Preparation.

The pasty mass left after alcohol-chloroform evaporation contained ascorbic acid in the combined form associated with other materials.

Purification could be effected by dissolving this crude ascorbigen preparation in a minimum quantity of water, centrifuging off the precipitate and evaporating the centrifugate to dryness over calcium chloride in a vacuum desiccator. The mass (A), thus obtained, is soluble in water and brown in colour. The p_H of the aqueous solution varied from 3.5 to 5.0 and it contained a small amount of free ascorbic acid. A considerable increase in dye value was obtained when the water extract was heated on a water-bath for 10 minutes in carbon dioxide atmosphere. In order to identify the released reducing substances, this material was subjected to the action of ascorbic acid oxidase, as described by Sen-Gupta and Guha (*loc cit.*). It was found that the crude ascorbigen preparation (A), corresponding to 1 kg. (roughly) of fresh cabbage, contained 7.43 mg. of reducing substances in combined state, of which non-specific reducing substances, estimated by the oxidase method, amounted to 3 mg. Therefore, ascorbic acid actually present in the combined state amounted to 4.43 mg. Thus about 40% of the reducing material was derived from a non-ascorbic acid complex. Further purification of the crude ascorbigen preparation (A) is in progress. The ascorbigen in preparation (A) was non-dialysable through parchment or cellophane membrane. When an electric current was allowed to pass through the ascorbigen solution using different membranes and at different p_H values no better concentration of ascorbigen was obtained. At low p_H values ascorbigen was slowly split up.

The elements detected by sodium fusion showed the presence of nitrogen and sulphur and no phosphorus in preparation (A). Nitrogen present varied from 11 to 13%. Preparation (A) did not respond to the xanthoproteic, biuret and Millon's tests. It gave positive glyoxylic acid and Pauly reactions. It reduced Fehling's solution and gave a very strong Molisch's test.

The yields of ascorbigen preparation (A) from cabbage were naturally different from different cabbage samples and varied from 0.7 g. to 1.5 g. per kg. of fresh cabbage, which gave yields of juice varying from 600 to 1000 c.c. by our method of extraction. 0.7-1.5 G. of ascorbigen preparation (A) contained 7-13 mg. of reducing substances in the combined state, of which about 40% consisted of non-specific reducing substances.

Further Concentration of Ascorbigen Preparation (A).

A partial purification was effected by tungstic acid precipitation. The ascorbigen preparation (A) containing 2.8 mg. reducing substances in the combined state was dissolved in a minimum quantity of water and centrifuged. The centrifugate was treated with 2 c.c. of 5% sodium tungstate.

Sulphuric acid (3 c.c. of 1.3 N) was then slowly added and the mixture was centrifuged. Excess of sulphuric acid was quickly removed from the centrifugate by baryta till the p_H was brought near 6.2, and excess of barium, if any, was removed by CO_2 . Alternatively, excess of sulphuric acid was removed by solid barium carbonate. The clear liquid obtained by centrifugation contained practically all the original ascorbigen present in preparation (A) and, on evaporation in a vacuum desiccator, gave a highly hygroscopic brownish mass. This also gave a strong Molisch's reaction.

CONCLUSION

Different protein-precipitating reagents were used for the concentration of ascorbigen from cabbage juice. Most of the ascorbigen, however, was obtained in the filtrate.

"Norite" charcoal and "active" charcoal (Schering-Kahlbaum, "carbo-active") were found to adsorb 60% of ascorbigen from fresh cabbage juice. Ascorbigen can be eluted in a large measure from this adsorbate by a chloroform-alcohol mixture.

The ascorbigen preparation obtained by the charcoal adsorption method gave a strong Molisch's reaction and reduced Fehling's solution. It contained nitrogen and sulphur but no phosphorus. It did not give xanthoproteic, biuret and Millon's tests. It gave positive glyoxylic acid and Pauly reactions.

The ascorbigen preparation on being split up by heat gave reducing substances, 60% of which were oxidised by ascorbic acid oxidase. The rest consisted of non-specific reducing substances, which were also apparently present in a combined state in the ascorbigen preparation.

The ascorbigen preparation could be further concentrated by means of sodium tungstate and sulphuric acid. The active materials were not precipitated by these reagents but appeared quantitatively in the filtrate.

Our best thanks are due to the Indian Research Fund Association for financing the researches on ascorbic acid.

DEPARTMENT OF APPLIED CHEMISTRY,
UNIVERSITY COLLEGE OF SCIENCE, CALCUTTA

Received September 4, 1939.

DISTRIBUTION OF FREE AND TOTAL ASCORBIC ACID IN THE LIVER AND MUSCLE OF BENGAL • FRESH-WATER FISH.

By K. C. SAHA.

The ascorbic acid contents of the liver and muscle tissues of 34 kinds of Bengal fresh-water fish have been determined by the usual method and by the method of Sen-Gupta and Guha

It has been shown by Rudra (*Biochem. J.*, 1936, **30**, 701) that of all parts of an animal generally used for edible purpose, the liver is the richest and the muscle the poorest in vitamin C, although considering all the tissues, the liver is not the organ richest in vitamin C. The concentration of the vitamin in the suprarenal cortex is greater than its concentration in the liver. But on account of the very minute size of the suprarenal in proportion to the liver, the latter is a greater source of the vitamin.

The present work was undertaken to investigate the ascorbic acid content of the liver and muscle tissues of some Bengal fresh-water fish and to find if any portion of the vitamin in these tissues is present in a combined form. It has been shown previously from this laboratory (Guha and co-workers, *J. Indian Chem. Soc.*, 1939, **16**, 481, 496) that many plant foodstuffs contain part of the ascorbic acid in a combined state, which has been called ascorbigen. Guha and Sen-Gupta (*Nature*, 1938, **141**, 974) also indicated that with certain animal tissues, for example with guinea-pig brain, a greater reducing value is obtained on heating in an atmosphere of hydrogen sulphide and its specificity is shown by the fact that the bulk of it is oxidised by ascorbic acid oxidase. In the present work, therefore, we have investigated the liver and muscle tissues of 34 kinds of Bengal fish by the method of Sen Gupta and Guha (*J. Indian Chem. Soc.*, 1939, **16**, 549; *Science and Culture*, 1938, **3**, 398; *Nature*, 1938, **141**, 974), which consists in determining the vitamin value after hot H_2S treatment and then finding which portion of this value disappears on treatment with ascorbic acid oxidase. We have called these the "true, total" ascorbic acid values. These values have been compared with those obtained by the usual technique of simple trichloroacetic acid treatment according to the method of Ghosh and Guha (*J. Indian Chem. Soc.*, 1935, **12**, 30).

EXPERIMENTAL.

Estimation of "true total" Ascorbic Acid.

The material (10 g.) under investigation was cut into small pieces and minced uniformly by passing through an 'Enterprise' hasher twice. The minced product was then disintegrated in a glass mortar with washed sea-sand after which the mixture was taken in a suspension of 50 c. c. of water and sulphuretted hydrogen passed in. After 5 minutes the suspension was heated under reflux on a boiling water-bath, while sulphuretted hydrogen was being passed. Heating was continued for 15 minutes and then sulphuretted hydrogen was removed completely (as tested by lead acetate paper) by a current of carbon dioxide. The suspension was then treated with 2.5 c.c. of 20% trichloroacetic acid, the mixture centrifuged and the filtrate made up to 100 c.c. This solution was titrated against 0.5 c.c. of *M/10-2:6*-dichlorophenol-indophenol to which 1 c.c. of glacial acetic acid had been previously added.

To estimate the reducing substances other than ascorbic acid, the same sample (10 g.) was treated with sulphuretted hydrogen as before and sulphuretted hydrogen was removed by carbon dioxide and centrifuged. After centrifuging, the volume of the filtrate was made up to 100 c.c. and 10 c.c. of the solution, thus obtained, were incubated with 10 c.c. of ascorbic acid oxidase solution of known potency (prepared from 400 g. of white gourd and dissolved in 250 c.c. of water) for $\frac{1}{2}$ hour at p_H 5.6 at 40° with the addition of 2 c.c. of acetate buffer. After the incubation period, the solution was titrated against 2:6-dichlorophenol-indophenol as usual. The difference between the two readings (with and without oxidase action) gave the "total true" ascorbic acid (Sen-Gupta and Guha, *J. Indian Chem. Soc.*, 1939, **16**, 549).

Free Ascorbic Acid by Simple Indophenol Titration.—The same sample of liver or muscle (10 g.), prepared in the way described before, was treated with 2.5 c.c. of 20% trichloroacetic acid; finely ground and taken up with 50 c.c. of water and centrifuged. The filtrate was made up to 100 c.c. and titrated against *M/10-2:6*-dichlorophenol-indophenol in the usual way.

The values given in Table I for each variety of fish are derived from estimations of 5-7 samples. The range of weights of the different individuals of each variety of fish is also given in the table.

DISCUSSION.

From the results it is clear that among all the different varieties of fish investigated *koi* (*Anabas testudineus*) has the highest ascorbic acid content

DISTRIBUTION OF FREE AND TOTAL ASCORBIC ACID, ETC. 513

TABLE I

Vitamin C in muscle and liver of Bengal fish.

Figures for ascorbic acid are given in mg. per 100 g. of the fresh tissue.

Ranges of body weight (in g.)	Bengali name of the fish	Zoological name	Vitamin C in muscle.		Vitamin C in liver	
			"Total true" vitamin.	Free vitamin	"Total true" vitamin	Free vitamin.
923-223	Kalbasu	<i>Labeo Calbasu</i>	10.6	9.1	64.8	60.2
28-41	Pati	...	15.3	13.7	40.1	37.6
111-325	Sarputi	<i>Barbus Sarana</i>	13.6	13.1	56.6	53.2
380-69	Vetki	<i>Lates Calctfer</i>	9.6	9.3	18.5	15.8
390-122	Pyrachanda	...	19.4	17.9	28.7	25.5
137-14	Tengra	...	17.6	18.8	54.2	47.6
75-22	Bacha	<i>Chptso magarua</i>	13.0	12.3	38.6	36.2
814-210	Ilisha	<i>Clupea ilisha</i>	24.1	24.0	53.9	50.2
75-22	Bata	...	negligible	negligible	7.3	6
18012-416	Catla	<i>Catla catla</i>	9.3	7.2	39.0	35.7
58-15	Koi	<i>Anabas testudineous</i>	32.0	31.6	67.8	65.3
45-20	Pakal	...	negligible	negligible	21.0	18.3
423-128	Bhola	<i>Sclaena cottor</i>	13.7	12.4	64.3	58.2
3150-236	Air	<i>Artus alius</i>	11.1	8.9	26.6	24.1
56-24	Fesha	...	negligible	negligible	17.6	16.1
1263-424	Sole	<i>Ophicephalus striatus</i>	9.2	7.6	36.7	32.2
516-56	Bele	<i>Glassgobtus glurits</i>	3.1	2.7	8.2	7.6
3-11	Maurala	<i>Amblypharyn godonmola</i>	5.2	5.0	19.2	17.8
518-69	Kujavetki	...	10.2	9.7	32.6	28.8
36-75	Singhi	<i>Saccobranchius fossilis</i>	9.3	8.8	56.3	49.7
46-72	Lata	<i>Ophicephalus punetatus</i>	negligible	negligible	19.7	17.9
11-31	Parsey	<i>Mugilparsia</i>	6.2	6.0	39.7	35.2
1327-316	Bhangar	...	12.0	11.6	56.9	47.2
303-78	Folui	<i>Notopterus notopterus</i>	5.6	4.8	21.6	18.3
209-72	Magur	<i>Clarius batrachus</i>	11.3	9.8	48.7	44.2
1120-103	Rohu (jheel)	<i>Labeo rohita</i>	23.2	22	171.1	163.7
8650-168	Rohu	"	20.1	18.6	158.9	152.5
1480-415	Boal	<i>Wallago attu</i>	7.6	6.1	20.4	18.3
912-126	Mrigal	<i>Cirrhinia mrigala</i>	14.8	12.6	32.3	29.2
150-45	Bam	..	3.2	3.1	11.3	9.8
6050-1530	Pangash	<i>Pangasius pangasius</i>	26.3	24.4	39.2	35.6
250-82	Bugda Chingri	...	6.5	6.3	54.1	47.4
11036-7320	Dhain	<i>Silonia Silundia</i>	18.8	16.0	127.4	116.3
119-48	Royna	...	7.6	5.3	11.8	10.2

in the muscle, viz., 30 mg. per cent. The next best muscle sources are *Pangash* (*Pangasius pangasius*) and *Rohu* (*Labeo rohita*). The concentration of the vitamin is highest in the Rohu fish liver, viz., 171.1 mg. and then comes *Dhain* liver (*Silonia Silundia*), viz., 127.4 mg. The difference between the total and free ascorbic acid is much greater in the case of liver than in muscle. It has also been found that both in the liver and muscle, the vitamin C concentration decreases as the size of the fish increases.

My best thanks are due to Prof. B. C. Guha for his advice and interest. The above work has been carried out under the auspices of the Indian Research Fund Association, to whom our best thanks are due.

DEPARTMENT OF APPLIED CHEMISTRY,
UNIVERSITY COLLEGE OF SCIENCE,
CALCUTTA UNIVERSITY.

Received September 4, 1939.

CONDENSATION OF ARSENIC CHLORIDE WITH DIALKYL AROMATIC AMINES.

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AND (MISS) K. M. YASHODA.

Arsenic trichloride has been condensed with methylethylaniline, dimethylnaphthylamine, dimethyl-*m*-toluidine and dimethyl-*p*-toluidine and the corresponding dialkylaminophenylarsenious oxides have been obtained. In the case of the first three compounds along with the arsenious oxides, tertiary arsenic compounds have also been obtained.

Arsenic trichloride condenses with dialkyl aromatic amines (Michelis and Rabinerson, *Annalen*, 1892, **270**, 139) at the *para* position with respect to the tertiary amino group with the formation of *p*-dialkylaminophenylarsenious chlorides, which were isolated as the corresponding arsenious oxides. In the case of dimethylaniline, the reaction proceeds further, one molecule of arsenic trichloride reacting with three molecules of the tertiary amine forming a triarylarsine compound {tri-*p*-dimethylaminophenylarsine, $[(CH_3)_2N \cdot C_6H_4]_3As$ }. Later on, it was observed that the propyl ether of phenylmethylglycine and amylaniline in pyridine solution underwent similar condensation with arsenic trichloride.

In this paper the reaction of arsenic trichloride has been extended to a few more dialkylaromatic amino compounds. Methylethylaniline, dimethyl- α -naphthylamine, dimethyl-*m*-toluidine and dimethyl-*p*-toluidine condense with arsenic trichloride forming dialkylaminophenylarsenious oxides. The first three amines, methylethylaniline, dimethyl- α -naphthylamine and dimethyl-*m*-toluidine give along with arylarsenious chlorides, small quantities of the triarylarsines as in the case of dimethylaniline, the arsenic group entering the *para* position with respect to the tertiary amino group and in the case of dimethyl-*p*-toluidine, where the *para* position is blocked, the condensation takes place in the *ortho* position.

The arsenious oxides have been converted into the corresponding chloride, bromide, iodide and arsenic acids.

The condensation of arsenic trichloride with methyl- and ethylbenzylanilines, dibenzylaniline and ethylbenzyl-*o*-toluidine does not proceed under similar conditions.

EXPERIMENTAL.

p-Methylethylaminophenylarsenious Oxide, $(EtMe)N \cdot C_6H_4 \cdot AsO$.—A mixture of methylethylaniline (15 g.) and arsenic chloride (25 g.) was

heated on a steam-bath for 2 hours. The resulting syrupy liquid was poured into cold water and the clear solution basified with sodium hydroxide solution (10 %) when some solid separated out which was removed. The filtrate was acidified with dilute hydrochloric acid and the *p*-methylethylaminophenylarsenious oxide precipitated by the addition of an aqueous solution of sodium carbonate. The compound (13 g.) was purified by dissolving in dilute hydrochloric acid and reprecipitating by sodium carbonate. (Found: N, 6.32; As, 33.68. $C_9H_{12}ONAs$ requires N, 6.22; As, 33.33 per cent). It is a white powder, easily soluble in chloroform and hot alcohol and sparingly soluble in ether and benzene and easily soluble in both dilute alkali and acids, m.p. 74-75°.

The *sulphide*, $(EtMe\cdot N)C_6H_4AsS$, prepared by passing dry hydrogen sulphide into a solution of the oxide, is insoluble in water, easily soluble in chloroform, carbon disulphide and acetone. It had m.p. 157° after crystallisation from chloroform. (Found: S, 13.31. $C_9H_{12}NSAs$ requires S, 13.29 per cent).

The *chloride hydrochloride*, $(MeEt\cdot N)C_6H_4\cdot AsCl_2\cdot HCl$ is a white amorphous hygroscopic powder, easily soluble in water, alcohol, acetone and chloroform, m.p. 99°. (Found: Cl, 33.33. $C_9H_{13}NCl_3As$ requires Cl, 33.62 per cent). Sodium carbonate converts it into the oxide.

The *bromide hydrochloride*, $(MeEt\cdot N)C_6H_4\cdot AsBr_2\cdot HBr$ is a hygroscopic powder, m.p. 143°. It is easily soluble in water and practically insoluble in ether. (Found: Br, 54.1. $C_9H_{13}NBr_3As$ requires Br, 54.48 per cent).

The *iodide hydrochloride*, $(MeEt\cdot N)C_6H_4\cdot AsI_2\cdot HI$ is a light yellow powder decomposing on exposure.

p-Methylethylaminophenylarsinic acid, $(MeEt\cdot N)C_6H_4\cdot AsO(OH)_2$, is a white amorphous powder, crystallisable from alcohol, and is soluble in chloroform, m.p. above 250°. (Found: As, 28.25. $C_9H_{14}O_3NAs$ requires As, 28.96 per cent).

Tri-p-methylethylaminophenylarsine, $[(MeEt\cdot N)C_6H_4]_3As$.—During the preparation of *p*-methylethylaminophenylarsenious oxide, the solids that separated on the addition of sodium hydroxide solution were treated with petroleum ether in which the unreacted amine dissolves leaving the triarylarsine 2.5 g. behind. These were washed repeatedly with petroleum ether and finally purified by crystallisation from chloroform, m.p. 206°. It is soluble in carbon disulphide and insoluble in ether, yield 2.5 g. (Found: As, 15.91. $C_{27}H_{36}N_3As$ requires As, 15.71 per cent). When equimolecular

quantities of methylethylaniline (5 g.) and arsenic trichloride (7 g.) were made to react, a better yield of triarylarsine (3 g.) was obtained.

1-*Dimethylaminonaphthyl* - 4 - *arsenious oxide*, $\text{Me}_2\text{N}\cdot\text{C}_{10}\text{H}_6\text{AsO}$, was obtained by mixing together dimethylnaphthylamine (10 g.) and arsenic trichloride (26 g.) and warming on a water-bath for 1 hour and the arsenious oxide isolated as in the preceding case. The substance (7 g.) is a white amorphous powder, readily soluble in chloroform, hot alcohol and carbon tetrachloride and sparingly soluble in ether, m.p. $98-100^\circ$. (Found : As, 28.5. $\text{C}_{12}\text{H}_{12}\text{ONAs}$ requires As, 28.71 per cent).

The *sulphide* is a fine yellow powder, insoluble in water and easily soluble in chloroform. m.p. 144° . (Found : S, 11.54. $\text{C}_{12}\text{H}_{12}\text{NSAs}$ requires S, 11.58 per cent).

The *chloride* is very easily soluble in water, m.p. $110-12^\circ$. (Found : Cl, 28.99. $\text{C}_{12}\text{H}_{12}\text{NCl}_3\text{As}$ requires Cl, 30.21 per cent).

The *bromide* is a white amorphous powder, easily soluble in organic solvents except in ether. (Found : Br, 49.14. $\text{C}_{12}\text{H}_{12}\text{NAsBr}_3$ requires Br, 49.38 per cent).

The *iodide* is a pale yellow powder, m.p. $119-20^\circ$. (Found : I, 60.36. $\text{C}_{12}\text{H}_{12}\text{NI}_3\text{As}$ requires I, 60.75 per cent).

Tri-(1-*dimethylaminonaphthyl*) - 4-*arsine*, $[(\text{Me})_2\text{N}\cdot\text{C}_{10}\text{H}_6]_3\text{As}$. — The triarylarsine (2 g.) was formed along with the arsenious oxide and was isolated as in the previous case. It is a white amorphous substance, sparingly soluble in ether and very soluble in chloroform and carbon tetrachloride, m.p. 148° . (Found : As, 12.46. $\text{C}_{30}\text{H}_{30}\text{N}_3\text{As}$ requires As, 12.8 per cent).

4-*Dimethylamino-2-methylphenylarsenious oxide*, $(\text{Me})_2\text{N}\cdot\text{C}_6\text{H}_3(\text{Me})\text{AsO}$, was obtained along with a little triarylarsine (1 g.) by mixing and warming on a water-bath dimethyl-*m*-toluidine (10 g.) and arsenic trichloride (25 g.) for 20 minutes, m.p. 108° . (Found : As, 33.09. $\text{C}_9\text{H}_{12}\text{ONAs}$ requires As, 33.33 per cent). The related *sulphide* had m.p. 137° .

4-*Dimethylamino-2-methylarsenic acid* does not melt below 250° . (Found : As, 28.71. $\text{C}_9\text{H}_{14}\text{O}_3\text{NAs}$ requires As, 28.96 per cent).

Tri-4-*dimethylamino-2-methylphenylarsine*, $[(\text{Me})_2\text{N}\cdot\text{C}_6\text{H}_3(\text{Me})]_3\text{As}$, is a white powder, soluble in chloroform and carbon disulphide, m.p. 98° . (Found : As, 15.58. $\text{C}_{27}\text{H}_{36}\text{N}_3\text{As}$ requires As, 15.7 per cent).

2-Dimethylamino-5-methylphenylarsenious oxide, $(\text{Me})_2\text{N}\cdot\text{C}_6\text{H}_3(\text{Me})\text{AsO}$, was prepared by warming dimethyl-*p*-toluidine (10 g.) and arsenic trichloride (25 g.) on the steam-bath for $1\frac{1}{2}$ hours, yield 4 g, m.p. $63\text{--}65^\circ$. (Found : As, 33.04. $\text{C}_9\text{H}_{12}\text{ONAs}$ requires As, 33.33 per cent).

Its *sulphide* had m.p. 68° . (Found : S, 13.32. $\text{C}_9\text{H}_{12}\text{NSAs}$ requires S, 12.29 per cent).

2-Dimethylamino-5-methylphenylarsinic acid, prepared in the usual way, is soluble in water. It does not melt below 250° . (Found : As, 28.69. $\text{C}_9\text{H}_{14}\text{O}_3\text{NAs}$ requires As, 28.96 per cent).

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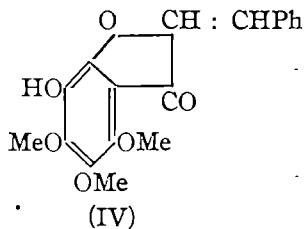
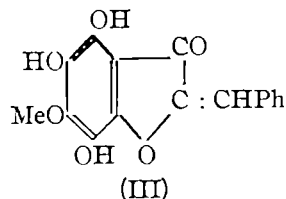
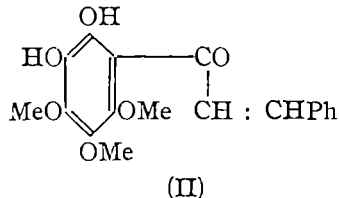
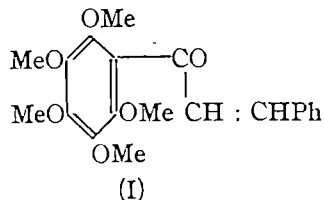
Received August 10, 1939.

THE CONSTITUENTS OF *DIDYMOCARPUS PEDICELLATA*.
PART IV. ISOLATION OF TWO NEW COLOURING
MATTERS AND THEIR RELATIONSHIP
TO PEDICIN.

BY SHARIFUDDIN WARSI AND SALIMUZZAMAN SIDDIQUI.

A second isomer of pedicin, pseudo-*isopedicin*, $C_{18}H_{18}O_6$, and a new colouring matter pedicidin, $C_{37}H_{36}O_{11}$, have been isolated from *D. pedicellata* and characterised. Pedicin can be converted into pseudo-*isopedicin* by Kostanecki's method and *isopedicin* was found to be transformed into pseudo-*isopedicin* on long storage. The mutual relationship of the three isomers of pedicin has been discussed. Pedicidin is probably a condensation product of the simpler colouring matters.

Sharma and Siddiqui (*J. Indian Chem. Soc.*, 1937, **16**, 1) have suggested the following tentative structures for pedicellin (I), pedicin (II), pedicinin (III) and *isopedicin* (IV).



The benzalcoumaranone structure for pedicinin was preferred to that of an isomeric flavanone because of its deep red colour and its colour reactions with ferric chloride, caustic alkali and sulphuric acid and because benzaldehyde was produced from it on treatment with alkali or permanganate. In view of the ease with which the five-membered ring in pedicinin ring is formed from the chalcone, pedicin, it appeared likely that *isopedicin* also should have a five and not a six-membered ring, hence it was deemed desirable to investigate this point more thoroughly by a closer study of pedicin and *isopedicin*.

With this object in view, the isolation of *isopedicin* in quantity was undertaken by a modified method in order to prevent its conversion to *pedicin* and also to facilitate the isolation of any other allied products, which might have been affected by the older method of separation (Siddiqui, *J. Indian Chem. Soc.*, 1937, **14**, 705).

On working up 2 kg. of the drug by the new method (*vide experimental*), the following two new products, along with the previously described colouring matters, were obtained.

(1) *Pseudo-isopedicin*, $C_{18}H_{16}O_3(OMe)_3$, m. p. 126-28°, yield 0.45 %

(2) *Pedicidin*, $C_{30}H_{18}O_4(OMe)_7$, m. p. 190°, yield 0.12 % The molecular formulae are proposed on the basis of their C-H, OMe and M. W. values.

Pseudo-isopedicin was found to be saturated to bromine and like *isopedicin*, is easily converted into *pedicin* on treatment with dilute alkali at ordinary temperature. *Pedicin* could be reconverted into *pseudo-isopedicin* by Kostanecki's method (*Ber.*, 1904, **37**, 784) of converting chalcones to flavanones (or to the corresponding coumaranones). A two year old sample of *isopedicin*, which was originally noted to melt at 105° (Siddiqui, *loc. cit.*) now melts at 127° and showed no depression on admixture with the natural *pseudo-isopedicin* or the product obtained from *pedicin* by Kostanecki's method. The melting point of *isopedicin* was recorded for the air-dried substance and the possibility of its having been a hydrate of the higher melting *pseudo-isopedicin* is now definitely excluded by the fact that the latter's m. p. is unaffected after crystallisation from dilute alcohol. *Pseudo-isopedicin* gives the same colour reactions as *isopedicin* with alkali, sulphuric acid and ferric chloride.

It is probable that *isopedicin* (m. p. 105°) is racemised into *pseudo-isopedicin* on long storage and naturally racemic product is obtained when a ring-closure of *pedicin* is effected by Kostanecki's method. A more detailed investigation, now possible because of the easy accessibility of *pseudo-isopedicin*, will decide between the flavanone or coumaranone structure. The results will be soon communicated.

In regard to the higher molecular product, *pedicidin*, it has been noted that it takes up bromine, gives nearly the same colour reactions as *isopedicin* with alkali, sulphuric acid and ferric chloride and is converted into a deep red crystalline compound (m. p. 163-66°) by the action of nitric acid. It is too early to express an opinion as to the exact nature of *pedicidin* but its colour reactions and the presence of seven methoxyls suggest that it is probably formed by the condensation of two molecules of the simpler colouring matters. A detailed investigation is in progress.

E X P E R I M E N T A L.

Isolation of Pedicidin and Pseudo-isopedicin.—Coarsely powdered leaves (2 kg.) were successively soxhletted with petroleum ether (A) and ether (B) and the two extracts worked up separately as follows.

The crude colouring matter, which crystallised out on cooling the concentrated ethereal extract was washed thoroughly with a concentrated solution of sodium carbonate and the ethereal mother-liquor (M) was first washed with sodium carbonate solution and then with dilute sodium hydroxide. The combined carbonate extracts from above were acidified and extracted with ether and finally with chloroform. The ether and chloroform extracts yielded pedicin as the least soluble fraction and crude *pseudo-isopedicin* was obtained from the mother-liquors of pedicin. The portion insoluble in carbonate forming the major fraction of the crude colouring matter yielded pedicin, while the dilute caustic alkali extract, mentioned above, gave residual pedicin on acidification and extraction with ether and free stearic acid (*vide* Part III of this series).

The ethereal mother-liquor (M), which after washing with dilute caustic alkali contained only neutral substances, was washed with dilute acid and then with water and worked up according to the method described for the corresponding fraction in Part I (*loc. cit.*, p. 705). The crude pedicellin was obtained mostly before and partly after the removal of the essential oil by steam distillation. The second colouring matter, pedicidin, was isolated from the least soluble fraction of crude pedicellin, by fractional crystallisations. The ether extract (B) thus gave pedicin (5g.), pedicin (4g.), pedicellin (6g.), more than half of *pseudo-isopedicin* (4.7g.), and the whole of the new product, pedicidin (2.5 g.). On being worked up in the above manner the petroleum ether extract (A) gave the whole of essential oils and fatty matter, the major part of pedicin (16 g.), about three-fourths of pedicellin (14 g.), very little of pedicin and nearly half of the total *pseudo-isopedicin* (4.3 g.). No pedicidin could be isolated from this extract.

Pure *pseudo-isopedicin*, m. p. 126°, was obtained from the crude product on repeated crystallisations from a mixture of acetone and petroleum ether, which removed the persistent traces of pedicin. Pedicidin was purified by successive crystallisations from chloroform, ethyl acetate and acetone.

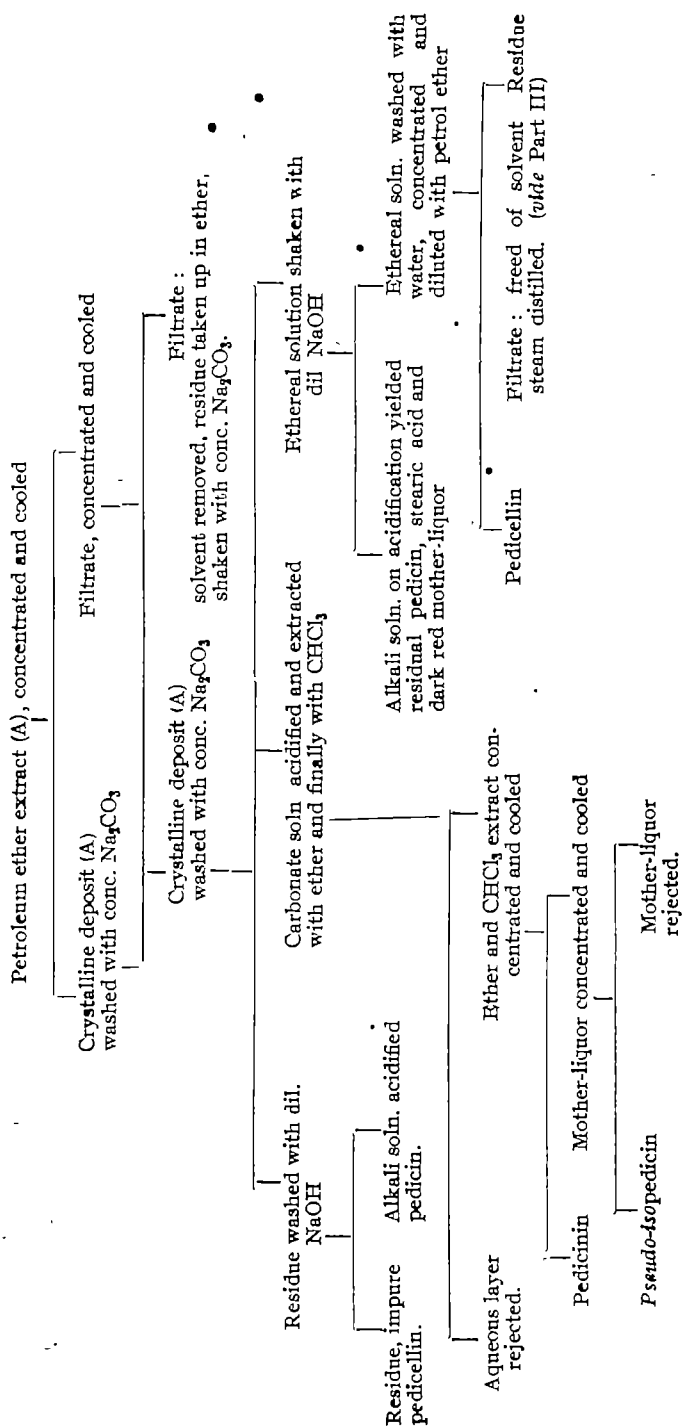
Pseudo-isopedicin, $C_{18}H_{18}O_6$, is easily soluble in alcohol, chloroform and benzene, fairly so in ethyl acetate and acetone, sparingly soluble in ether and nearly insoluble in petroleum ether. It crystallises in straw coloured prismatic rods which dissolve in ammonia or in a

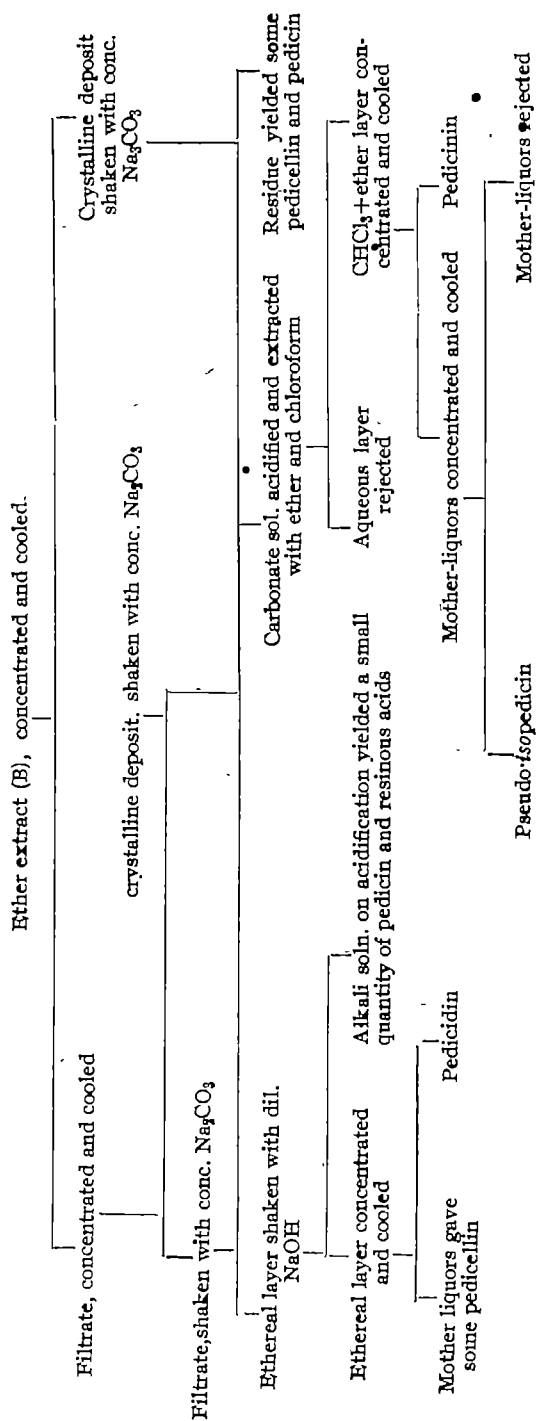
concentrated sodium carbonate solution with a yellow colour. In dilute sodium hydroxide it dissolves with a golden yellow colour, which soon turns brownish red and ultimately to deep red on standing. The change of colour from golden yellow to deep red evidently indicates its conversion to pedicin which takes place more rapidly in a 10% solution of sodium hydroxide. With sulphuric acid it gives deep red colour which vanishes on dilution. With ferric chloride its alcoholic solution gives an evanescent pale green colouration, which changes *via* light brownish red to deep reddish brown on standing. [Found : C, 65.44; H, 5.56; OMe, 27.54; M.W. (Rast), 320. $C_{15}H_{16}O_3(OMe)_3$ requires C, 65.5; H, 5.5; OMe, 28.1 per cent. M.W., 330).

Conversion of Pedicin into Pseudo-iso-pedicin.—A mixture of pedicin (0.38 g.) in alcohol (5 c.c.) and hydrochloric acid (d 1.16, 2.5 c.c.) was refluxed for about 18 hours on the steam-bath. After removal of most of the alcohol, the mixture was diluted with water and extracted with ether and the ethereal solution washed with dilute sodium carbonate. The carbonate extract on acidification and extraction with ether yielded a small quantity of a reddish product which was not further examined. The ethereal layer yielded a light reddish yellow viscous mass, which on being successively treated with ether and cold acetone gave pale yellow prismatic rods (ca 0.2 g.), m.p. 125–26°, undepressed by *pseudo-isopedicin* and unaltered by crystallisation from dilute alcohol.

Pedicidin, $C_{37}H_{38}O_{11}$, is insoluble in ether and petroleum ether, soluble in chloroform, acetone and ethyl acetate in the hot and crystallises from these solvents in pale yellow elongated prisms, m. p. 190°. It gives a light brownish red reaction in alcohol and gives a reddish brown solution in concentrated sulphuric acid. [Found after drying at 100° in *vacuo* over P_2O_5 : C, 68.08; H, 5.77; OMe, 33.85 M. W. (Rast), 731, (cryoscopic), 747; $C_{30}H_{16}O_4(OMe)_7$ requires C, 67.69; H, 5.48; OMe, 33.08 per cent. M.W., 656).

On addition of concentrated nitric acid to its glacial acetic acid solution it develops a dark red colour which quickly deepens, taking a violet tinge. The reaction mixture was immediately diluted with ice-water and extracted with ether; the residue from the ethereal solution crystallised on rubbing with alcohol, yielding deep red prismatic rods which after repeated washings with acetone on a porous plate, melted at 164–66° (shrinking from 161°).





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Received August 16, 1939.

CHEMISTRY OF SPIRO-COMPOUNDS. PART I. PREPARATION OF CYCLOPENTANE-SPIRO-CYCLOPENTANONE AND CYCLOHEXANE-SPIRO-CYCLOHEPTANONE.

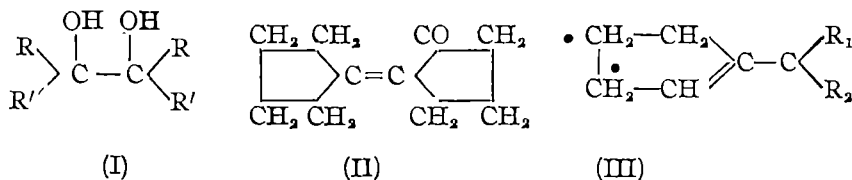
BY MUHAMMAD QUDRAT-I-KHÜDA AND AMALENDU KUMAR RAY.

By the reduction of *cyclopentanone* and *cyclohexanone* with magnesium and aluminium amalgam *dicyclopentane-diol* and *dicyclohexane diol* respectively have been prepared. These diols are accompanied by a certain proportion of *cyclopentylidene-cyclopentanone* and *cyclohexylidene-cyclohexanone*. *Dicyclopentane-diol* on being treated with sulphuric acid undergoes pinacolone pinacol transformation and gives *cyclopentane-spiro-cyclohexanone*. This on being oxidised yields *cyclopentane-1-carboxy-1-butyric acid*, which yields *cyclopentane-spiro-cyclopentanone*. *Dicyclohexane-diol* gives a mixture of *cyclohexane-spiro-cycloheptanone* and *dicyclohexene*.

The study of the chemistry of simple spiro-compounds holds out very interesting possibilities. Regarding the nature of these rings, a wealth of information may be available by the determination of the heat of combustion of the hydrocarbons themselves. For the synthesis of these compounds attention was directed towards the preparation of some of the ketones which could then be easily transformed into the required hydrocarbons.

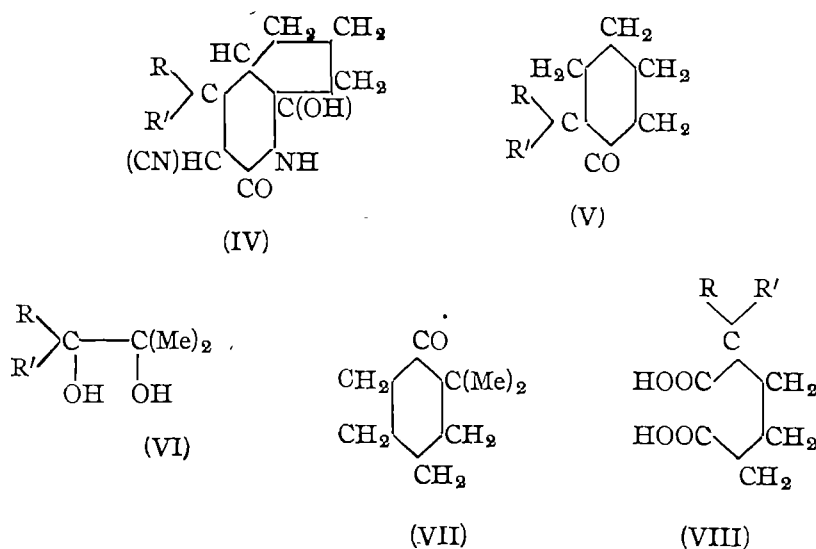
The spiro-ketone, *cyclopentane-spiro-cyclohexanone* was prepared by Meiser (*Ber.*, 1899, **32**, 2049). It was considered desirable to adopt a similar method for the synthesis of other spiro-compounds. The yield of *dicyclopentane-1:1'-diol* (I) by the method of Meiser, being very meagre, the yield was appreciably increased by the method of Gruber and Adams (*J. Amer. Chem. Soc.*, 1935, **57**, 2555) and this was further improved by substituting aluminium amalgam for magnesium according to the method of Barnett and Lawrence (*J. Chem. Soc.*, 1935, 1104). Along with the diol (I), in earlier experiments, with magnesium amalgam, we isolated a large proportion of *cyclopentylidene-cyclopentanone* (Wallach, *Ber.*, 1896, **29**, 2963). In later experiments its quantity was smaller, nevertheless it could be isolated in a fairly appreciable quantity. The ketone was actually a mixture of *cyclopentenylcyclopentanone* (III) and *cyclopentylidenecyclopentanone* (II). An iodometric estimation showed that it consisted of about 25 % of the $\beta\gamma$ - isomeride, the rest being the $\alpha\beta$ -unsaturated compound. The

ketone (II) condensed readily with cyanoacetamide yielding the product (IV).



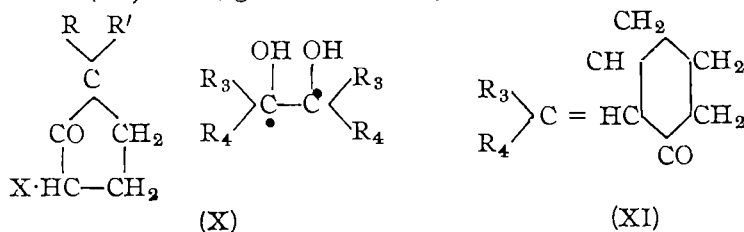
where $\text{RR}' = \text{C}_4\text{H}_9$ and $\text{R}_1\text{R}_2 = \text{C}_4\text{H}_9\text{O}$.

The conversion of the diol (I) into the spiro-ketone (V) was almost quantitative, and it could be ranked with the conversion of *cyclopentane-isopropyl*-diol (VI) into 1:1-dimethyl*cyclohexan-2-one* (VII) (Meerwein *Annalen*, 1910, 376, 152). The spiro-ketone (V) is easily oxidised with nitric acid, giving a mixture of acids from which *cyclopentane-1-carboxy-1-butyric acid* (VIII) could be isolated in predominantly larger quantity, the other constituent being succinic acid. Thus it is to be inferred that only a small fraction of the compound underwent secondary decomposition. The ketone (V) gives only a monobenzylidene derivative suggesting the presence of one methylene group by the side of the carbonyl group.



The ester derived from (VIII), on being acted upon by sodium ethoxide gives the keto-ester (IX, $\text{X} = \text{CO}_2\text{Et}$), which on hydrolysis and decarboxylation produces *cyclopentane-spiro-cyclopentanone* (IX, $\text{X} = \text{H}$). The

ketone (IX, X=H) gives a monobenzylidene derivative with benzaldehyde.

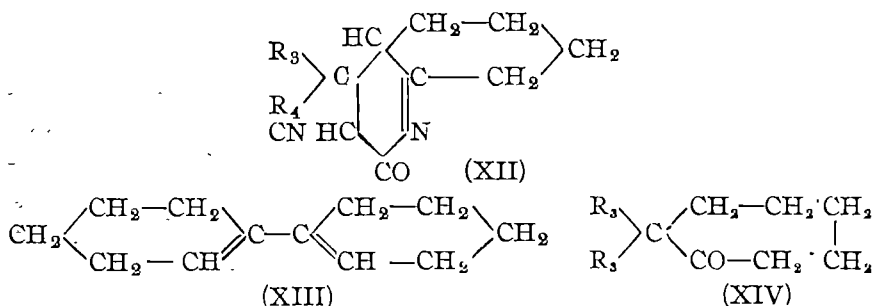


where $\text{R}_3\text{R}_4 = \text{C}_5\text{H}_{10}$.

Dicyclohexane-diol (X) could also be prepared under similar conditions from cyclohexanone, being accompanied by quite an appreciable amount of cyclohexenylcyclohexanone (XI). The constitution of the ketone (XI) has been established by the formation of a semicarbazone and also by its giving the compound (XII) with cyanoacetamide.

The pinacol-pinacolone transformation with dicyclohexane-diol gives a ketone with a very characteristic camphoraceous odour, accompanied by a large percentage of the unsaturated hydrocarbon $\Delta^{1:1'}$ -dicyclohexene (XIII) (Wallach and Pauly, *Annalen*, 1911, 885, 95).

The ketonic compound should be represented as cyclohexane-spiro-cycloheptanone (XIV). The ketone has been converted into its semicarbazone and it gives a benzylidene compound with great ease.



The small yield of the ketone (XIV) is rather disheartening. When a sufficient quantity of the material is obtained, it is expected to utilise it for the preparation of cyclohexane-spiro-cyclohexan-2-one in a manner analogous to the formation of compound (IX).

EXPERIMENTAL.

Dicyclopentan-1:1'-diol (I).—cyclopentanone (90 c.c.) containing mercuric chloride (13.5 g.) was added in a thin stream to boiling benzene (100 c.c.) containing magnesium powder (12 g.), in a 3-litre flask, which was heated on the water-bath. After refluxing on the water-bath for 1 hour, water (30 c.c.) was added and heating continued for 1 hour more. The ben-

zene solution was then filtered and the residue extracted with 50 c.c. of boiling benzene. The solvent from the united filtrates was removed under reduced pressure and to the residual oily liquid enough petroleum ether (b. p. 30° - 50°) was added and the mass was cooled with ice when the solid diol (12 g.) separated out fairly completely. From the mother-liquor, the solvent was distilled off and the residue was fractionated under reduced pressure when a further crop of diol (2.3 g.) was obtained on its distillation together with an oily liquid which boiled at 108° - 110° /5mm. The liquid has been identified as *cyclopentylidenecyclopentanone* which yields a semicarbazone, m.p. 224° (cf. Kon and Nutland, *J. Chem. Soc.*, 1926, 3101). The diol crystallised from petroleum (b. p. 70 - 80°) as colourless needles, m.p. 109° . (Found: C, 70.29; H, 10.88. $C_{10}H_{18}O_2$ requires C, 70.58; H, 10.58 per cent).

The yield was very much improved by substituting aluminium powder in place of magnesium (cf. Barnett and Lawrence, *loc. cit.*) under the following conditions. *cyclopentanone* (300 g.), aluminium powder (66 g.), mercuric chloride (31 g.) and dry benzene (400 c.c.) were heated on the water-bath for 2 hours. Water (250 c.c.) and some more benzene (450 c.c.) were then added and the heating was continued for 3 hours more. The diol was then worked up as in the previous case giving a yield of 66 g. The by-product, *cyclopentylidenecyclopentanone*, is produced in this case also, though not mentioned by Barnett and Lawrence.

Pinacolin Rearrangement of Dicyclopentane-1:1'-diol: Formation of cyclopentane-spiro-cyclohexan-2-one.—The dicyclopentane-diol (20 g.) and sulphuric acid (20%, 150 c.c.) were heated together in a flask fitted with distillation arrangements at 120 - 125° for 2 hours and the distillate was collected. The spiroketone was then extracted with ether and the ethereal extract, after washing with sodium carbonate solution and water was dried over sodium sulphate. The ether was then removed and the spiroketone collected at 120° /45 mm., yield 16 g. The *semicarbazone* of the ketone was obtained quite readily, m.p. 189 - 90° (cf. Clemo and Ormston, *J. Chem. Soc.*, 1933, 352). (Found: C, 63.04; H, 9.15. $C_{11}H_{18}ON_3$ requires C, 63.15; H, 9.09 per cent).

Benzylidene Derivative.—The spiroketone (5 g.), benzaldehyde (8 g.), rectified spirit (120 c.c.) and 10% caustic soda solution (30 c.c.) were mixed together and stirred for 3 days. The mixture was diluted with water; extracted with ether, the solvent was then distilled off and the residue freed from benzaldehyde by steam. The residual mass was taken up in ether and the extract, after being washed with 10% caustic soda solution and water was dried over anhydrous sodium sulphate. On removing the solvent the residual oil solidified within a short time, yield 5 g. It crystallised from petrol

(b.p. 30-50°) in pale yellow needles, m.p. 75°. (Found : C, 84.7; H, 8.3. $C_{17}H_{20}O$ requires C, 85.0; H, 8.3 per cent).

Oxidation of cyclopentane-spiro-cyclohexan-2-one to cyclopentane-1-carboxy-1-butyric Acid (VIII).—The spiro-ketone (18 g.) was gradually added from a dropping funnel to a warm mixture of nitric acid (45 c.c.) and water (12 c.c.) heated on the water-bath. When the reaction was over, the flask was heated on the water-bath for $\frac{1}{2}$ hour more and left overnight. The solution was evaporated on the water-bath with addition of water until nitric acid was completely driven off. The mass was then taken up in ether, dried, and the solvent removed. The residue was kept in a vacuum desiccator for several days when the acid partially solidified and weighed 19 g. The pure acid was obtained from the ester on hydrolysis. It was recrystallised from hydrochloric acid, m.p. 92°; (mixed m.p. with the synthetic acid prepared by Qudrat-i-Khuda and Mukherjee, *vide* second Part, this issue, p. 525).

Ethyl cyclopentane-1-carbethoxy-1-butyrate was prepared from the acid (36 g.) in absolute alcohol (230 c.c.) in the presence of hydrogen chloride as usual. It distilled at 140-142°/6 mm., yield 29 g. (Found: C, 65.79; H, 9.18. $C_{14}H_{24}O_4$ requires C, 65.62; H, 9.37 per cent). It had $d_4^{32.1}$, 1.017; n_D , 1.449. Found : $[R_L]_D$, 67.59 (calc. 67.96). A small quantity of an ester was obtained as a lower boiling fraction (90-93°/6 mm.) which yielded succinic acid on hydrolysis.

Ethyl cyclopentane-spiro-cyclopentan-2-one-3-carboxylate (IX, X = CO₂Et).—To a cold solution of sodium (1.8 g.) in absolute alcohol (50 c.c.) the dibasic ester (20 g.) was added and the mixture refluxed on the water-bath for 6 hours. The alcohol was then distilled off and water poured into the cooled residue. The solution was neutralised with hydrochloric acid, and the separated insoluble oily layer extracted with ether, the ethereal extract was washed with water and dried over anhydrous sodium sulphate and the ether removed. The keto-ester distilled at 127°/5 mm., yield 11 g. (Found: C, 68.20; H, 8.76. $C_{12}H_{18}O_3$ requires C, 68.57; H, 8.58 per cent). It had $d_4^{33.8}$, 1.033; n_D , 1.451. Found : $[R_L]_D$, 54.75 (calc. 54.88).

cyclopentane-spiro-cyclopentan-2-one (IX, X = H).—The keto-ester (11 g.) was heated on the sand-bath with 10% hydrochloric acid for 4 hours. The product was extracted with ether, the extract washed with sodium carbonate solution and water and dried over anhydrous sodium sulphate. It distilled at 115°/32 mm., yield 7 g. (Found: C, 77.80; H, 10.13. $C_9H_{14}O$ requires C, 78.26; H, 10.14 per cent). It had $d_4^{32.1}$, 0.9891; n_D , 1.471. Found: $[R_L]_D$, 39.1 (calc. 39.4). The ketone readily gave a semicarbazone,

m.p. 214° . (Found: C, 59.46; H, 9.6. $C_9H_{17}ON_3$ requires C, 59.01; H, 9.28 per cent)

The *benzylidene derivative*, prepared in the same manner as in the case of *cyclopentane-spiro-cyclohexan-2-one*, crystallised in pale yellow plates from petroleum ether (b.p. $30-50^{\circ}$), m.p. 64° . (Found: C, 84.5; H, 7.6. $C_{16}H_{18}O$ requires C, 84.9; H, 7.9 per cent).

Dicyclohexane-1:1'-diol (X) was prepared under the same experimental conditions as in the case of compound (I) using magnesium amalgam, yield 15 g. from 98 g. of *cyclohexanone*, while by using aluminium instead, 58 g. of the diol could be obtained from 200 g. of *cyclohexanone*. It crystallised from petrol (b.p. $70-80^{\circ}$), m.p. 130°

Pinacol-pinacolin Transformation of Dicyclohexane-1:1'-diol.—The pure diol (40 g.) and sulphuric acid (50%, 300 c.c.) were warmed on the water-bath for 2 hours when the reaction was complete. The whole thing was chilled, diluted with ice-water and then extracted with ether. The ethereal extract was washed with sodium carbonate solution and water and then dried over anhydrous sodium sulphate, ether was removed and the product (30 g.) was collected at $110-114^{\circ}/6\text{mm}$. It consisted of a mixture of $\Delta^{1:1'}$ -dicyclohexene and *cyclohexane-spiro-cycloheptanone*.

Treatment of the Above Mixture with Semicarbazide Acetate.—Semicarbazide hydrochloride (30 g.) and sodium acetate (40 g.) were dissolved in the minimum quantity of water and to this solution the above mixture (30 g.) was added. To this was added enough methyl alcohol and the solution heated on the water-bath for 1 hour. The alcohol was then distilled off and the solution cooled in ice, when the semicarbazone of *cyclohexane-spiro-cycloheptanone* separated out. The semicarbazone was filtered off and washed with petrol and then with water, the petrol washing being added to the original filtrate. The filtrate was extracted with petrol. From the petroleum extract, the solvent was removed and the residue was again treated with semicarbazide acetate as before to remove the last trace of ketone. After filtration of the semicarbazone, the aqueous portion was again extracted with petrol and the petroleum extract furnished an oily liquid, b.p. $111^{\circ}/6\text{mm}$. The oil was identified as $\Delta^{1:1'}$ -dicyclohexene. (Found: C, 88.47; H, 10.86. $C_{12}H_{18}$ requires C, 88.88; H, 11.11 per cent).

The semicarbazone of *cyclohexane-spiro-cycloheptanone* was crystallised from methyl alcohol, m.p. $216-17^{\circ}$, yield 5g. (Found: C, 66.3; H, 9.3. $C_{13}H_{23}ON_3$ requires C, 65.8; H, 9.7 per cent).

Regeneration of cyclohexane-spiro-cycloheptan-2-one from its Semicarbazone.—The thrice crystallised semicarbazone (7 g.) and hydrochloric acid (10%, 70 c.c.) were warmed together on the water-bath for 4 hours,

when an oily layer separated out. The oil was extracted with ether and the ethereal extract was washed with sodium carbonate solution and water and dried over anhydrous sodium sulphate. The solvent was then removed and the ketone was distilled at $120^{\circ}/8$ mm., yield 5g. (Found: C, 79.76; H, 10.9. $C_{12}H_{10}O$ requires C, 80.0; H, 11.1 per cent). It had $d_4^{33.8}$, 0.9795; n_D , 1.483. Found $[R_L]_D$, 52.64 (calc. 53.22).

The *Benzylidene derivative* crystallised in pale yellow needles from petrol (b.p. $30-50^{\circ}$), m.p. 123° . (Found: C, 85.2; H, 7.9. $C_{18}H_{22}O$ requires C, 85.7; H, 8.2 per cent).

Condensation of cycloHeptylidene-cycloheptanone with Cyanoacetamide.—To the sodio derivative obtained from cyanoacetamide (4.2 g.), and sodium (1.14 g.) in the minimum quantity of alcohol, the ketone (7.5 g.) was added and the mixture refluxed on the water-bath for 3 hours. The alcohol was then distilled off, water was poured into the residue and the mass acidified with hydrochloric acid and the precipitate was crystallised several times from dilute ethyl alcohol, when a white crystalline substance was obtained, m.p. $215-16^{\circ}$, yield 6 g. (Found: C, 67.15; H, 7.98. $C_{13}H_{18}O_2N_2$ requires C, 66.66; H, 7.69 per cent).

Condensation of cycloHexenylcyclohexanone with Cyanoacetamide.—The method was precisely the same as in the condensation of *cyclopentylidene-cyclopentanone* with cyanoacetamide. From 4.5 g. of the ketone, 4 g. of the condensation product were obtained. The product was crystallised from glacial acetic acid, m.p. same as given by Sen and Neyogi (*J. Indian Chem. Soc.*, 1930, 7, 305) who effected the condensation in a different manner. (Found: C, 73.55; H, 8.52. $C_{16}H_{20}ON_2$ requires C, 73.77; H, 8.19 per cent).

The grateful thanks of one of us (A.K.R.) are due to the Director of Public Instruction, Bengal, for the award of a research scholarship during the tenure of which this work was completed.

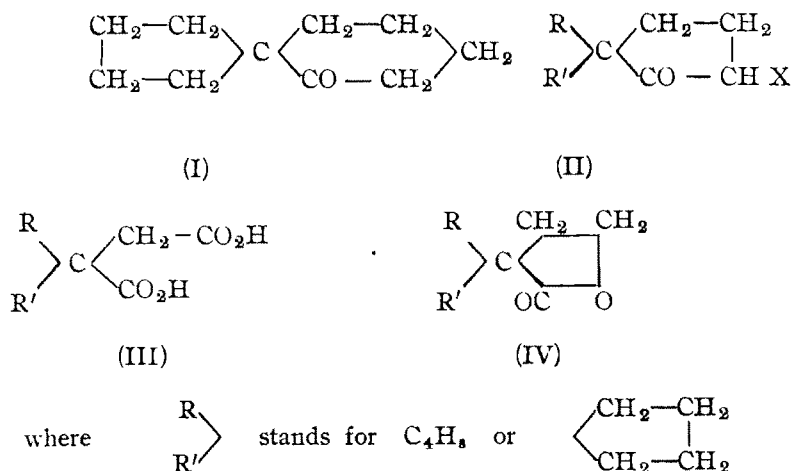
CHEMISTRY OF SPIRO-COMPOUNDS. PART II. SYNTHESIS OF CYCLOPENTANE-SPIRO- CYCLOPENTANONE.*

BY MUHAMMAD QUDRAT-I-KHUDA AND ASUTOSH MUKHERJEE.

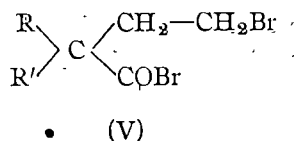
cyclopentane-spirocyclopentanone has been synthesised from *cyclopentane-1-carboxy-1-butyric acid*, which has been synthesised in a way which leaves no doubt regarding its configuration. The lactone obtained by the reduction of the anhydride of *cyclopentane-1-carboxy-1-acetic acid* yields a bromo-ester which by the action of sodiummalonic ester and subsequent hydrolysis gives *cyclopentane-1-carboxy-1-butyric acid*. This acid has been converted into the spiro-ketone both by Dieckmann condensation of its ester, followed by hydrolysis, as also by its pyrogenetic decomposition in presence of baryta.

cyclopentane-spiro-cyclopentanone (II, X=H) was prepared by Qudrat-i-Khuda and Ray (*J. Indian Chem. Soc.*, 1939, **16**, 518) from *cyclopentane-spiro-cyclohexanone* (I) through its product of oxidation, *viz.*, *cyclopentane-1-carboxy-1-butyric acid* (VII). The constitution of this ketone has been finally settled by an independent synthesis described in this paper.

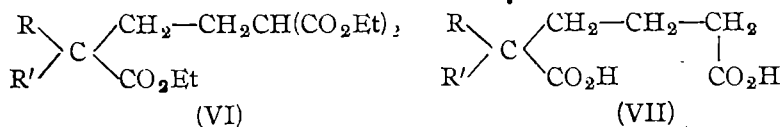
When the anhydride of *cyclopentane-1-carboxy-1-acetic acid* (III) is reduced by alcohol and sodium, it is converted into the lactone (IV).



The lactone ring is opened up with phosphorus pentabromide when a bromo-acid bromide (V) is obtained, which is converted into the bromo ester by the action of alcohol.



The bromo-ester reacting with sodio malonic ester, furnishes the tricarbethoxy compound (VI). On hydrolysis, this tricarbethoxy compound is converted into *cyclopentane-1-carboxy-1-butyric acid* (VII) which is found to be identical with the acid obtained previously (*J. Indian Chem. Soc.*, 1939, **16**, 518.)



The adipic acid (VII) gives the corresponding ester which after Dieckmann condensation yields the keto-ester (II, X=CO₂Et), which is hydrolysed to the ketone (II, X=H). *cyclopentane-spiro-cyclopentanone* could also be obtained by the dry distillation of the *cyclopentane-adipic acid* (VII) in presence of barium hydroxide.

EXPERIMENTAL.

Anhydride of cyclopentane-1 carboxy-1-acetic acid was prepared by heating the mixture of the acid (100 g.), acetic anhydride (75 c. c.) and acetyl chloride (15 c. c.). It was then distilled under reduced pressure when the anhydride boiled at 174°/38 mm., yield 80 g. It was kept in a vacuum desiccator for 2 days over fused calcium chloride and caustic potash.

Reduction of the Anhydride.—A solution of the anhydride (40 g.) in absolute alcohol (250 c. c.) was quickly dropped on the sodium kept in a 2-litre flask from a dropping funnel by a Y-tube, the other end of the Y-tube being connected to an inverted reflux condenser ending in a calcium chloride guard tube. The temperature of the bath was maintained at 80-90° during the addition of alcohol. When the solution of the anhydride had been added, absolute alcohol (350 c.c.) was poured on and the temperature of the bath was then raised to 130° and kept there for 1 hour more, when the whole of the sodium went into solution. It was then cooled and water (250 c.c.) was added and the alcohol was removed by distilling in a current of steam. The solution was then acidified by hydrochloric acid, cooled and extracted by ether, the ethereal layer was washed with sodium bicarbonate solution, water and dried. The solvent was removed and the product distilled at 154°/40 mm. (Found: C, 68.4; H, 8.5. C₉H₁₂O₂)

requires C, 68.5; H, 8.5 per cent). It had $d_4^{30.1}$, 1.0751; n_D , 1.46345. Found: $[R_L]_D$, 35.9 (calc. 36.3). The yield of the lactone was 80 g. from 120 g. of the anhydride. From the bicarbonate extract about 35 g. of succinic acid could be recovered after the treatment of this whole amount of the anhydride.

Preparation of Ethyl cyclopentane-1-bromoethyl-1-carboxylate.—Phosphorus pentabromide (270 g.) was added to the above lactone (80 g.) and the mixture heated on a water-bath for 2 hours. The product was then cooled in ice and slowly poured into well cooled absolute alcohol (200 c.c.) and shaken, care being taken to avoid any moisture. It was then left at the ordinary temperature overnight, poured into crushed ice and the separated oil was taken in ether and washed thoroughly with a solution of sodium bicarbonate and water and dried. It was finally distilled at $118^\circ/5$ mm., yield 62 g. (Found: Br, 32.1. $C_{10}H_{17}O_2Br$ requires Br, 32.6 per cent). It had $d_4^{30.1}$, 1.2481; n_D , 1.46816. Found: $[R_L]_D$, 55.46 (calc: 55.48).

Ethyl cyclopentane-1-carbethoxy-1- α -carbethoxy-butyrate (VI).—The above bromo-ester (55 g.) was allowed to drop on to sodiomalonic ester (prepared from 32 g. of malonic ester using 4.6 g. of sodium), taken in a three-necked flask provided with a mercury-seal stirrer, under stirring at the ordinary temperature and was left overnight. Next day it was heated on a water-bath for 3-4 hours, and then the alcohol was removed. The cold mass was diluted with water and acidified with hydrochloric acid and the precipitated oil was taken up in ether and the ethereal solution washed with sodium bicarbonate and water and finally dried. The residue after removal of the solvent boiled at $154^\circ/6$ mm. and consisted of the ester (VI). (Found: C, 62.09; H, 8.48. $C_{17}H_{28}O_6$ requires C, 62.19; H, 8.5 per cent). It had $d_4^{31.1}$, 1.05401; n_D , 1.44486. Found: $[R_L]_D$, 82.7 (calc. 83.1).

cyclopentane-1-carboxy-1-butyric Acid (VII).—The above tricarboxylic ester (25 g.) was hydrolysed with concentrated hydrochloric acid (100 c.c.) by heating on a sand bath for 14-16 hours. A thick oily residue remained which was taken up in ether and washed completely with a solution of sodium carbonate. The sodium carbonate extract was acidified and extracted again with ether. After removal of the solvent the acid solidified in a vacuum desiccator and crystallised from ether-petroleum mixture, m.p. 92° . [Found: C, 59.85; H, 8.0; M.W. (by titration), 200.1. $C_{10}H_{16}O_4$ requires C, 60.0; H, 8.0 per cent. M. W., 200]. The *di-anilide* of the acid was obtained from aniline and the acid chloride, prepared by the action of thionyl chloride on the free acid. It crystallised from dilute alcohol, m.p. 163° . (Found: C, 75.5; H, 7.4. $C_{22}H_{26}O_2N_2$ requires C, 75.1; H, 7.4 per cent).

Ethyl cyclopentane-1-carbethoxy-1-butyrate.—*cyclopentane-1-carboxy-1-butyric acid* (45 g.) was dissolved in absolute alcohol (200 c.c.) and the solution saturated with dry hydrogen chloride. It was then left overnight and next it was heated for 4 hours. After usual methods of purification, the ester was obtained as a mobile liquid which boiled at $140^{\circ}/6$ mm., yield 35 g. (Found: C, 65.8; H, 9.2. $C_{14}H_{24}O_4$ requires C, 65.6; H, 9.4 per cent). It had $d_4^{22.1}$, 1.0170; n_D , 1.44910. Found: $[R_L]_D$, 67.67 (calc. 67.96).

Ethyl cyclopentane-spiro-cyclopentane-2-one-3-carboxylate (II, X = CO_2Et) was obtained by the action of metallic sodium dissolved in alcohol on ethyl cyclopentane-1-carbethoxy-1-butyrate. The mixture was left in ice for $\frac{1}{2}$ hour and was then heated on a water-bath for 6 hours. The mass was then cooled and the keto-ester isolated in the usual way. It was distilled at $135^{\circ}/9$ mm. (Found: C, 68.2; H, 8.8. $C_{12}H_{18}O_3$ requires C, 68.6; H, 8.6 per cent). It had $d_4^{23.8}$, 1.03301; n_D , 1.45111. Found: $[R_L]_D$, 54.75 (calc. 54.88).

cyclopentane-spiro-cyclopentane-2-one (II, X = H). (Method I)—Ethyl cyclopentane-spiro-cyclopentane-2-one-3-carboxylate (10 g.) was heated on a sand-bath for 4 hours with hydrochloric acid (10%, 50 c.c.). It was then cooled and extracted with ether, the ethereal extract washed with a solution of sodium bicarbonate and water and finally dried over fused magnesium sulphate. The solvent was then removed and the spiro-ketone distilled at $115^{\circ}/30$ mm. It gave a semicarbazone, m.p. 215° . (Found: C, 61.5; H, 8.7. $C_{10}H_{14}ON_2$ requires C, 61.5; H, 8.7 per cent). The ketone regenerated from the semicarbazone boiled at $115^{\circ}/30$ mm. (Found: C, 78.09; H, 10.09. $C_8H_{14}O$ requires C, 78.26; H, 10.14 per cent). It had $d_4^{20.1}$, 0.9790; n_D , 1.46603. Found: $[R_L]_D$, 39.1 (calc. 39.4).

(Method II). A mixture of cyclopentane-1-carboxy-1-butyric acid (7 g.), and baryta (1 g.) and iron powder (1 g.) was heated in a 100 c.c. Jena distilling flask at first at 150° for 3 hours and then at 290° – 340° for 2 hours when a liquid product distilled over. It was taken up in ether and the ethereal solution purified by usual means. The residual ketone was converted into its semicarbazone, m.p. 215° (mixed m.p.).

KINETICS OF THE REACTION BETWEEN POTASSIUM PERSULPHATE AND THE ALKYL IODIDES. PART I. INFLUENCE OF SOLVENTS, ACIDS AND SALTS.

BY M. S. TELANG AND V. V. NADKARNY.

The solvent effect of alcohols, acids and the corresponding esters on the kinetics of the persulphate-alkyl iodide reaction, alternates in ascending the homologous series. This effect runs parallel with their dipole moments. A qualitative relationship between the specific catalytic effect of the cations, H^+ , K^+ , NH_4^+ and Na^+ and their transport numbers is suggested. The chloride ion influences the kinetics much more than the corresponding SO_4^{2-} or NO_3^- . Amongst mineral acids, HNO_3 , HCl , H_2SO_4 and H_3PO_4 , the order of catalytic influence runs parallel with that of their ionisation constants although the parallelism is by no means quantitative.

Menschutkin (*Z. physikal. Chem.*, 1890, **5**, 589) in studying the triethylamine-ethyl iodide reaction in a large number of solvents observed a qualitative relationship between the dielectric constant of the medium and its influence on the reaction velocity. Grimm, Ruf and Wolff (*Z. physikal. Chem.*, 1931, **13 B**, 301) in making a further examination of the Menschutkin reaction observed striking changes in passing through the following solvents, diphenyl ether, diphenylamine and diphenylmethane with $>O$, $>NH$ and $>CH_2$ groups respectively. Richardson and Soper (*J. Chem. Soc.*, 1929, 1873) have given two empirical rules for solvent influence. Whenever there is a definite solvent effect, cohesion and polarity of solvents play an important part in influencing a reaction. In the present investigation to compare the relationship between the dipole moments of the solvents and the reaction velocity the following types of solvents are chosen: alcohols ($R-OH$), acids ($R-COOH$) and esters ($R_1-CO.O.R_2$).

"Primary kinetic salt effect" is observed with neutral alkali salts. In moderate concentrations of these univalent ions Brönsted-Debye-Hückel equation, $V = k \cdot C_A \cdot C_B \cdot (1 + \mu \cdot \beta_s)$ is obeyed up to at least $\mu = 1.6$. The values of F , the kinetic activity factor, have been calculated from the straight line graphs plotted from the reaction velocity against μ , the ionic strength.

Preliminary experiments have indicated that the conditions under which the kinetic measurements have been made are quite convenient for comparison and the reaction was found to be monomolecular.

E X P E R I M E N T A L.

Ethyl iodide was chosen as the alkyl iodide for all the comparable results in this work. Ethyl iodide was redistilled after drying with calcium chloride and preserved in the dark in contact with ignited silver powder, over calcium chloride in brown dropping bottles. The weight of a single drop was calculated by determining the weight of 20 drops. A normal solution of ethyl iodide in absolute alcohol was made by taking the required number of drops of ethyl iodide, and then the strength of the iodide was checked by Stepanow's and Volhard's methods. The solution could not be preserved for more than two to three days without decomposition of the iodide taking place.

Recrystallised potassium persulphate was dried at room temperature in a vacuum over sulphuric acid. The purity of the persulphate was estimated by the ferrous sulphate method. The salts were of "Kahlbaum" purest quality and were used without further purification.

The temperature of the thermostat was maintained at $50^{\circ} \pm 0.1^{\circ}$ by means of Ostwald toluene-mercury electrical regulator, used in conjunction with an electrically driven stirrer. Solutions were made at thermostat temperature.

The iodine liberated during the reaction serves as a measure of the progress of the reaction. The reaction was arrested by dipping the flask containing the reaction-mixture in ice-cold water. The reaction was carried out in suitable round-bottom flasks fitted with efficient reflux condensers. After two hours' heating and subsequently arresting the reaction, the reaction-mixture was shaken in a separating funnel with benzene and the iodine present was completely extracted. The solution of iodine in benzene was directly titrated against 0.005N-sodium thiosulphate, the end-point being indicated by the complete decolourisation of benzene. The titration was carried out in a glass-stoppered bottle. At first the presence of the iodine in the benzene solution was apparent on account of its deep colour and gentle rotation of the liquid caused sufficient mixing with the thiosulphate. Towards the end of the titration, the bottle was stoppered and shaken after each addition of sodium thiosulphate and the end-point was reached when the solution was completely decolourised.

The velocity constants were calculated from the equation,

$$k = \frac{2.302}{t} \log \frac{a}{a-x}$$

a , being the initial concentration of the ethyl iodide and the persulphate (which are taken in equivalent proportions) in g. equiv. per litre and x ,

the number of g. equiv. per litre of iodine liberated in t minutes. All determinations recorded were at 50°.

For each determination, the procedure was as follows.—

0.2*N*-Alcoholic solution (10 c.c.) of ethyl iodide, 0.2*N*-solution of potassium persulphate (10 c.c.) and solvent (5 c.c.) were made up to 100 c.c. with freshly distilled water, in a 100 c.c. measuring flask. The whole quantity of the reaction mixture was transferred to a round-bottom flask fitted with an efficient reflux condenser and lowered into the thermostat.

After two hours' heating, the reaction was arrested by dipping the flask in ice-cold water. If there was any iodine adhering to the walls of the condenser-tube, it was washed down into the reaction flask with benzene and the titration was carried out as described above.

For the determination of the salt effect, the reaction mixture consisted of 10 c.c. of 0.2*N*-ethyl iodide, 10 c.c. of 0.2*N*-potassium persulphate and x c.c. of 4*N*-salt solution, made up to 100 c.c. with distilled water.

TABLE I.

Solvent.	Vol. of N/200 iodine titrated.	$k \times 10^6$.	Dipole moment $\mu \times 10^{18}$.
<i>Alcohols.</i>			
Methyl	9.8 c.c.	207.10	1.67
Ethyl	7.9	165.00	1.72
<i>n</i> -Propyl	3.5	72.93	1.65
<i>n</i> -Butyl	5.1	105.50	1.74
<i>Acids.</i>			
Formic	0.8	15.35	1.4
Acetic	8.0	166.90	
Propionic	4.8	99.74	
Butyric	5.4	111.30	
<i>Esters.</i>			
Ethyl formate	0.6	9.82	1.4
acetate	5.3	109.30	
propionate	2.5	49.87	
butyrate	6.2	128.50	
Methyl acetate	6.9	143.90	1.8
Ethyl	5.3	109.30	
<i>n</i> -Propyl	2.2	44.12	
<i>n</i> -Butyl	2.8	57.54	

TABLE II.

Vol. of 4 N-NaCl added.	Vol. of N/200 iodine titrated	Ionic concentration.	Velocity constant $k \times 10^6$.
0 c.c.	7.7 c.c.	0	161.2
2.5	13.0	0.1 N	274.3
5	15.8	0.2	335.7
10	25.0	0.4	537.1
20	37.7	0.8	824.9

TABLE III.

20 c.c. of 4N-R-Cl,	Vol. of N/200 iodine titrated.	ionic concentration	Velocity constant $k \times 10^6$	Kinetic activity factor (F)
HCl	71.2 c.c.	0.8 N	1632.0	8.588
KCl	44.6	0.8	984.0	5.178
NH ₄ Cl	38.7	0.8	847.8	4.462
NaCl	37.7	0.8	824.9	4.361

TABLE IV.

4N salt (20 c.c.).	N/200-I ₂ titrated.	Ionic conc.	$k \times 10^6$
NaCl	37.7 c.c.	0.8 N.	824.9
NaNO ₃	16.7	0.8	354.9
Na ₂ SO ₄	16.3	0.8	347.2
KCl	44.6	0.8	984.0
†K ₂ SO ₄	16.6	0.8	353.0
CuSO ₄	30.05	0.8	648.3
Fe(NO ₃) ₃	55.2	0.8	1237.0

TABLE V.

4 N Na ₂ SO ₄	N/200-I ₂ titrated	Ionic conc.	$k \times 10^6$.
2.5 c.c.	10.0 c.c.	0.1 N	211.0
5	10.8	0.2	228.2
10	14.0	0.4	295.4
20	16.3	0.8	347.2
40	26.9	1.6	387.6

† 80 c.c. of N-K₂SO₄ were taken as it is not possible to prepare 4 N solution.

TABLE VI.

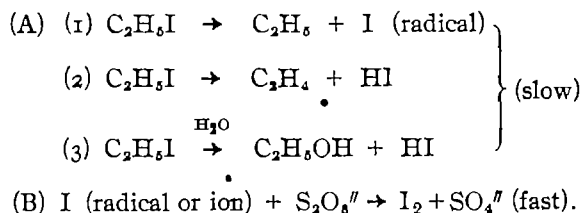
4N H ₂ SO ₄	N/200-I ₂ titrated.	Ionic conc.	$k \times 10^6$.
2.5 c.c.	19.8 c.c.	0.1 N	422.0
5	30.8	0.2	667.0
10	44.85	0.4	989.7
15	64.25	0.6	1457.0
20	69.5	0.8	1591.0
25	77.9	1.0	1805.0

TABLE VII.

20 c.c. of 4N acid, i.e., 0.8N ionic conc.	N/200-I ₂ titrated	$k \times 10^6$.
HNO ₃	112.3	2747.0
HCl	71.2	1632.0
H ₂ SO ₄	69.5	1591.0
H ₃ PO ₄	25.3	544.8

DISCUSSION.

The reaction was found to be monomolecular. The reaction could be formulated in one of the following ways:—



Further work regarding the mechanism of the reaction is in progress.

To illustrate the parallelism between the alteration in the dipole moment of solvent and the reaction velocity, the moments of the alcohols in the homologous series are given. To compare the general solvent influence for three types of solvents—alcohols, acids and esters, the average values of the dipole moments are considered in Table I, dipole moment being a function of the polar group only, irrespective of the remainder of the molecule. However, the variations in the moments of the individual members in the same series are never very considerable.

Examining the values of the velocities in different solvents in a general manner, it is observed that the rates are in the order, acids > alcohols > esters, and the average dipole moments are in the reverse order, acids (1.4) < alcohols (1.7) < esters (1.8). (The values for the first members are neglected on account of possible induction effects.) It may be unjustifiable to draw a general conclusion with such limited data, but it does not appear improbable that the rates in this reaction and the polarity of solvents chosen are in the reverse order. Further investigations on the lines of Soper *et al* (*J. Chem. Soc.*, 1929, 1873; 1931, 2297; 1935, 1393; *Proc. Roy. Soc.*, 1933, **A** 140, 59, 71) are being carried out by us and will be published later on.

In order to ascertain the specific effects of different cations on the velocity of the reaction, experiments were carried out in solutions containing separately equivalent concentrations of potassium, ammonium, sodium and hydrogen ions, all being univalent. The velocity constants observed are given in Table III. For solutions of the same ionic strength, the values are highest in the presence of hydrogen ions and lowest in that of sodium. The reaction is so sensitive to hydrogen ions, that in more concentrated solutions of H_2SO_4 , deviations from the linear relationship is at once evident. This is probably due to the decreasing ionisation of H_2SO_4 with increasing concentration of the acid. Similar results have been found for

the monobromoacetate-thiosulphate reaction (La-Mer, *Chem. Reviews*, 1932, **10**, 199), the mono-iodo acetate reaction (Holmberg, *Z. physikal. Chem.*, 1921, **97**, 134), and for the persulphate-iodide reaction (Howells, *J. Chem. Soc.* 1939, 463). Observations were extended to include the anions of potassium and sodium and cations of copper and iron, by the addition of equivalent amount of their ions. The two cations are known to catalyse the persulphate-iodide reaction to a very great extent. It can be observed from Table IV that the chlorides of potassium and sodium are far more active in accelerating the reaction than the corresponding nitrate or sulphate and practically there is no difference in the activity of nitrate and sulphate ions. In the case of chlorides, probably the iodide from the alkyl iodide is interchanged and hence there is a marked effect on the velocity of the reaction.

The velocity constant-ionic strength curves ($k-\mu$) plotted from data in Tables II, V and VI converge at low ionic strengths. The extrapolated value for k at zero ionic strength, i.e., k_0 is approximately 190×10^{-6} , the experimental value 161.2×10^{-6} is less than the former. The extrapolated value is used for calculating the relative kinetic activity factors, F , (Table III) from the equation $k = k_0 F$ (*vide* Brönsted, *Z. physikal. Chem.*, 1922, **102** 169) k_0 has the same value whatever cations are present. The salt effect is in the sequence $H^+ > K^+ > NH_4^+ > Na^+$. The order of the activity coefficients of the salts employed is $HCl > NaCl > KCl$, etc. and the sequence of the viscosities of the chloride solutions of the same salts is $K^+ < Na^+ < H^+$, etc. Neither of these two sequences can be compared with that of the salt effect observed in this reaction, although in the persulphate-iodine reaction, Howells (*loc. cit.*) finds parallelism between the viscosities and the specific effects of cations. The velocities observed in this reaction decrease in the sequence of the transport numbers of the cations. (For the values of the transport numbers, see Washburn, *J. Amer. Chem. Soc.*, 1909, **31**, 322; Washburn and Millard, *ibid.*, 1915, **37**, 694.) On account of the hydration (solvation) of the ions, the size of the ion-hydrate complex decreases in passing down the series, $Na^+ > NH_4^+ > K^+ > H^+$, and presumably, the speeds of the ions increase from Na^+ to H^+ . It appears that this order can be correlated with that of the specific catalytic effect of the cations employed.

Amongst mineral acids, the order is as follows: $HNO_3 > HCl > H_2SO_4 > H_3PO_4$. This order runs parallel with their ionisation constants and in the case of HNO_3 , the velocity of the reaction is highly influenced. It was suspected whether HNO_3 acts as an additional oxidising agent besides

BIOLOGICAL VALUE OF THE PROTEINS OF SOME SPECIES OF BENGAL FISH BY THE NITROGEN BALANCE AND GROWTH METHODS.

By K. P. BASU AND K. GUPTA.

The biological value of the proteins of some common species of Bengal fish, viz., Katla (*Catla catla*), Milgel (*Channa mrigala*), Air (*Arius arius*), Kot (*Anabas Testudineus*), Singhi (*Saccobranchius Fossilis*), Sarputi, Poa and Koral has been determined by the nitrogen balance as well as by the growth methods. The digestibility and biological value of the fish proteins are in general very high. The digestibility varies between 83 and 97% cent. and the biological value between 70 and 88%. A growth per g. of proteins ingested varies from 1.48 to 1.83 per day. •

Fish constitutes an important part of the dietary in Bengal and in view of the fact that the nutritive value of proteins of pulses, an important source of proteins for Indians is not very high, the determination of the biological values of the proteins of Bengal fish, which are taken almost daily in moderate amounts, is a very important problem. The biological values of the proteins of two very common varieties of Bengal fish, the *Labeo rohita* (*ruhee*) and the *Clupea ulisa* (*hilsa*) were previously determined in this laboratory (Basu and De, *Indian J. Med. Res.*, 1938, 23, 177).

In the present paper the biological values of several other common species of Bengal fish have been investigated both by the balance sheet and growth methods.

EXPERIMENTAL.

Composition and the Preparation of Fish and Fish meal.—The head, tail, scales and bones of the fish were rejected. The fish was cut into thin slices and put in the steam oven. When the slices were dry, they were chopped in a small chopper and finally dried in the sun.

In the case of fish without scales (except *Singhi*) the outer deposit of fat was also rejected.

The following is the list of the different varieties of fish with their scientific and Bengali names and the percentages of protein, fat and moisture in the whole fish and in the dried meal. The percentage of proteins in the whole fish generally varies between 16 and 21, that

of moisture between 68 and 75 and that of fat between 0.4 and 3.4 excepting *Koi* which contains 8.8% fat and 14.8% protein.

Bengali names.	Scientific names.	Fish meals			Whole fish.		
		Crude protein.	Ether extract	Moisture	Crude protein.	Fat	Water
Katla	<i>Catla catla</i>	73.5%	12.27%	7.2%	19.2%	2.5%	70%
Mrigal	<i>Cirrhina mrigala</i>	78.36	4.02	7.46	19.6	1.0	74
Air	<i>Arius arius</i>	77.85	16.88	4.9	20.5	2.2	68
Koi	<i>Anabas testudineus</i>	61.79	28.59	3.16	14.6	8.8	71
Singhi	<i>Saccobranchus fossilis</i>	81.44	7.04	6.88	16.0	1.1	76
Sarputi	.	70.7	18.11	5.84	17.5	2.0	74
Poa	..	75.42	10.55	5.77	18.6	1.7	75
Koral	...	92.98	1.42	3.11	21.0	1.5	72

Composition of the Diet.—The nitrogen-free diets were of the same composition as those used by previous workers in this laboratory (*loc. cit.*).

The diets containing the fish proteins were prepared by replacing the necessary amounts of starch in the nitrogen-free diet to the fish meals, which had been previously freed from fat.

Balance Sheet Method.—The technique was the same as that followed by the previous workers in this laboratory (Basu, Nath and Ghani, *Indian J. Med. Res.*, 1936, 23, 789, 811; Basu, Nath and Mukherjee, *ibid.*, 1937, 24, 1001; Basu and De, *ibid.*, 1938, 26, 177).

Typical data of the balance sheet method are given in Tables I and II. The results are summarised in Table III.

TABLE I.

Experiment with nitrogen-free ration.

(Figures of intake and excretion represent daily averages).

Rat No.	Average weight.	Food intake.	Urinary N.	Faecal nitrogen.	
				Total.	per g. of food intake.
506	232 g.	9.2 g.	49.9 mg.	19.6 mg.	2.13 mg.
507	224.5	9.9	58.9	11.9	1.2
508	227	9.9	46.1	13.38	1.35
509	219	10.0	49.6	24.2	2.42
510	222.5	9.25	48.9	21.5	2.32
511	235.5	8.4	45.3	15.1	1.80

TABLE II.
Biological value of Katla fish protein (10% level).
(Figures of intakes and excretion represent daily averages).

Rat No.	Average body weight.	Intake		Faecal nitrogen		Food N absorbed.		Urinary nitrogen		Food N utilised.	B. V.	Mean • D. B. V.	
		Food.	Nitrogen.	Total.	Endo.	Exo.	Total.	Endo.	Exo				
506	218 g	9'73g.	148'2 mg	41'03 mg.	20'72 mg.	20'31 mg.	127'89 mg.	78'44 mg.	49'9 mg.	28'54 mg.	99'35 mg.	77'68	86'29
507	215'5	9'08	138'4	28'46	10'9	17'56	120'84	88'82	58'9	29'92	90'92	75'24	87'1
508	223	10'55	160'7	37'04	14'24	22'80	137'9	76'24	46'1	30'14	107'76	78'14	85'81
509	210'5	10'0	152'4	47'92	24'2	23'72	128'68	75'04	49'6	25'44	103'24	80'23	84'44
510	208'5	9'25	141'0	37'72	21'46	16'26	124'74	78'43	48'9	29'53	95'21	76'33	88'47
511	226'5	11'35	173'0	43'64	20'44	23'20	149'89	75'32	45'3	30'02	119'87	80'11	86'6

TABLE III.

Biological value (B.V.) and digestibility (D) of the proteins of some Bengal fish (at 10% level).

Rat No.	Average body wt.	Katla		Mrigel		Air		Sarputi	
		B.V.	D.	B.V.	D.	B.V.	D.	B.V.	D.
501	225 g.					76'28	93'52	85'24	97'19
502	208					75'06	95'43	80'48	95'33
503	210'5					72'65	96'33	81'07	93'94
504	221					74'05	93'22	83'72	96'47
505	222'5					74'8	92'12	80'11	98'02
506	232	77'68	86'29			73'97	95'30	83'87	96'77
507	224'5	75'24	87'1						
508	227	78'14	85'81						
509	219	80'23	84'44						
510	222'5	76'33	88'47						
511	235'5	80'11	86'6						
512	234								
513	243'5			72'1	92'8				
514	241'5			74'24	89'84				
515	226'5			70'57	92'44				
516	236			69'55	90'36				
517	214			71'49	94'42				
518	216'5			74'79	94'32				
Average.		77'96	86'45	72'12	92'36	74'47	94'32	82'42	96'29

Rat No.	Average body wt.	Koi		Singh		Poa		Koral	
		B.V.	D.	B.V.	D.	B.V.	D.	B.V.	D.
501	225 g.					76'05	96'54	80'5	98'5
502	208					74'94	97'66	85'22	95'23
503	210'5					77'2	94'5	83'6	95'7
504	221					75'51	97'21	82'95	96'18
505	222'5					75'3	93'4		
506	232								
507	224'5	84'24	93'74						
508	227	88'17	92'82						
509	219	85'65	97'55						
510	222'5	88'3	95'4						
511	235'5	84'9	97'86						
512	234	89'78	97'43						
513	243'6			91'6	97'1				
514	241'5			85'64	96'35				
515	226'5			86'72	93'27				
516	236			87'5	97'05				
517	214			90'81	93'2			85'8	97'72
518	216'5			88'43	95'82			81'81	98'1
Average		86'84	95'6	88'45	95'47	75'8	95'86	83'3	97'07

TABLE IV.

Biological value of proteins of fish by growth method (at 15 per cent level).

Material.	No. of rats.	Period of expt. weeks.	Increase in wt. Variation.	Mean.	Total food intake Variation.	Mean	Protein intake (Mean).	B.V. = $\frac{\text{Gain in wt.}}{\text{Protein intake}}$
Kalia (<i>Catla catla</i>)	6	$\begin{cases} 4 \\ 8 \end{cases}$	71'5-88'5 g. 106-132	79'3 g. 119'1	227'4-252'4 g. 368'8-504'2	240'4 g. 437'4	36'06 g. 65'61	2'20 1'83
Air (<i>Artus artus</i>)	6	$\begin{cases} 4 \\ 8 \end{cases}$	55'5-89'5 82'5-125	70'0 105'7	174'6-286'8 305'6-478'9	237'2 420'8	35'58 63'12	1'97 1'67
Mrigel (<i>Cirrhmia mrigala</i>)	5	$\begin{cases} 4 \\ 8 \end{cases}$	50-62 67-94'5	55'9 80'7	168'2-196'8 259'8-359'9	182'8 310'6	27'42 46'5	2'04 1'73
Singhi (<i>Saccobranchias fossilis</i>)	6	$\begin{cases} 4 \\ 8 \end{cases}$	45-63'5 61'5-92'5	50'8 74'5	171'4-220'5 297'1-385'4	183'5 233'6	27'52 35'04	1'84 1'48

Growth Method.—The biological value of proteins of four fish (*Katla*, *Air*, *Mrigel* and *Singhi*) was determined by this method, the technique employed being the same as that used by previous workers in this laboratory (*loc. cit.*). The results are given in Table IV.

DISCUSSION.

In the following table the biological value, digestibility at 10% protein level and also the growths induced in young rats per g. of protein intake at 15% level of different fish proteins have been collected.

TABLE V.

Fish (Bengali name).	Scientific name.	Digestibility.	Biological value at 10% level.	Growth per g. of protein intake at 15% level per day.
Katla	<i>Catla catla</i>	86.45	77.96	1.83
Mrigel	<i>Citrhina mrigala</i>	92.38	72.12	1.73
Air	<i>Arius arius</i>	94.32	74.47	1.67
Sarputi		96.29	82.42	...
Koi	<i>Anabas testudineus</i>	95.6	86.84	...
Singhi	<i>Saccobranchus fossilis</i>	95.47	88.45	1.48
Poa	.	95.86	75.8	...
Koral	...	97.07	83.3	...
Ruhee	<i>Labeo rohita</i>	88.6	78.9	1.71
Hilsa	<i>Clupea tilisa</i>	82.6	69.5	1.48

It would appear from the above that except in the case of *Hilsa*, the digestibility and biological value of the fish proteins are very high. The small fishes *Koi* and *Singhi* possess very high biological values and these species are also recommended for invalids in our country. The growth-promoting qualities of the *Singhi* are, however, not as good as those of the *Ruhee*, *Katla*, *Mrigel* and *Air*. The *Hilsa* also possesses comparatively poor growth-promoting properties.

The different fishes contain a large percentage of proteins of very good quality and their consumption as a daily article of food by the Bengalees is a very good habit.

THE ESTIMATION OF VITAMIN C IN FOODSTUFFS.

BY PRATUL NATH SEN-GUPTA AND B. C. GUHA.

The previous method of estimating ascorbic acid in food-stuffs after heating the aqueous suspensions in H_2S (Sen-Gupta and Guha, *J. Indian Chem. Soc.*, 1937, **14**, 95) has been modified by the introduction of treatment with ascorbic acid oxidase, which appears to give more accurate values for "total, true" ascorbic acid. Values obtained by this method are higher than those obtained by the Tillmans-Harris method. The stability of ascorbic acid oxidase has been investigated.

The simple trichloroacetic acid extraction method has been extensively employed for the estimation of vitamin C in foodstuffs by titration with 2 : 6-dichlorophenol-indophenol. Along with vitamin C in plant food-stuffs, the enzyme, ascorbic acid oxidase, has been found to be widely distributed (Chakraborty and Guha, *Indian J. Med. Res.*, 1937, **24**, 839). This enzyme is capable of oxidising ascorbic acid into the reversibly oxidised form (dehydroascorbic acid) when the plant tissue is disintegrated. If the grinding of the tissue is carried out under trichloroacetic acid, the action of the oxidase is minimised but not wholly suppressed. The amount of ascorbic acid which is thus oxidised cannot reduce the dye but is biologically potent. The simple Tillmans-Harris method, therefore, would tend to give a value for total ascorbic acid, which is a little too low. At the same time, evidence has been given (Sen-Gupta and Guha, *J. Indian Chem. Soc.*, 1939, **16**, 496) pointing to the presence of combined ascorbic acid or ascorbigen in several plant tissues, which is biologically potent but would not reduce the dye unless split up by heating. Thus the estimation of total ascorbic acid should include (a) the free ascorbic acid, (b) the dehydroascorbic acid which may be formed by the action of the oxidase, and (c) ascorbigen, if any. We, therefore, described in a previous paper (Sen-Gupta and Guha, *J. Indian Chem. Soc.*, 1937, **14**, 95) a method involving the heating of an aqueous suspension of the tissue in H_2S , so as to reduce the dehydroascorbic acid and split up ascorbigen, followed by titration against the indophenol indicator, so as to get the value of the total ascorbic acid.

The question, however, arises whether all the reducing material produced by heating in H_2S is ascorbic acid. This may be investigated by

using van Eekelen's method (Emmerie and van Eekelen, *Biochem. J.*, 1934, **28**, 1153) to remove interfering substances. But we have found in unpublished experiments that if known quantities of ascorbic acid are added to extracts of several varieties of foodstuff, like cabbage, onion, guava and *patol* (*Trichosanthes dioica*), van Eekelen's method enables only about 60-75 per cent of the added ascorbic acid to be estimated. This shows that, while eliminating interfering substances, mercuric acetate at the same time causes some loss of ascorbic acid. But, even if van Eekelen's method is applied to cabbage and *patol* after H_2S treatment in the hot and in the cold, a difference is obtained in the reducing value (Sen-Gupta and Guha, 1939, *loc. cit.*). Thus, although van Eekelen's method involves some loss of ascorbic acid, the application of this method also shows that heat produces some reducing substance, which, as we have shown earlier (Sen-Gupta and Guha, 1939, *loc. cit.*), consists largely of ascorbic acid as tested with ascorbic acid oxidase. Part of the reducing substances produced by heating in H_2S , however, is non-specific. It is, therefore, apparent that the previous method for the estimation of ascorbic acid by heating an aqueous suspension of the foodstuff in H_2S (Sen-Gupta and Guha, 1937, *loc. cit.*) would give rather high values for ascorbic acid. The method has, therefore, been modified by introducing the action of ascorbic acid oxidase after treatment with H_2S in the hot condition. A preliminary note on the subject has been published elsewhere (Sen-Gupta and Guha, *Science and Culture*, 1938, **3**, 398).

The ascorbic acid oxidase has been prepared both from cucumber and white gourd (Ghosh and Guha, *J. Indian Chem. Soc.*, 1937, **14**, 725) and its stability investigated under different conditions of storage. This information is necessary if the oxidase is to be used as a reagent for routine determination of vitamin C. Biological experiments are in progress to see how far the values obtained by this method agree with those obtained biologically.

EXPERIMENTAL.

Ascorbic acid oxidase was prepared for these experiments from cucumber by the method of Tauber *et al.* (*J. Biol. Chem.*, 1935, **110**, 271; see also Ghosh and Guha, *J. Indian Chem. Soc.*, 1937, *loc. cit.*) and purified by precipitating twice from aqueous solution by acetone. The oxidase preparation takes some time to dissolve in water.

The following plant foodstuffs were investigated—cabbage, *patol* (*Trichosanthes dioica*) and onion. All of them were treated in a similar

way. The same sampled foodstuff, was taken for each of the following experiments.

(a) 10 G. were extracted with trichloroacetic acid in the usual way (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935, **12**, 30). An aliquot was titrated against the indophenol indicator and another aliquot titrated after treatment with ascorbic acid oxidase.

(b) 10 G. were disintegrated, suspended in 50 c.c. of water and treated with H_2S for 30 minutes. H_2S was removed in a current of CO_2 or N_2 and then the mixture was treated with 2.5 c.c. of 20 per cent trichloroacetic acid. After centrifugation the volume was made up to 100 c.c., aliquots of which were titrated severally before and after oxidase treatment.

(c) 10 G. were disintegrated, suspended in 50 c.c. of water, treated with H_2S for 15 minutes in the cold and then heated for 15 minutes in H_2S on the water-bath. After removal of H_2S by a current of CO_2 or N_2 the mixture was treated as under (b).

In all the methods (a), (b) and (c), the amount of reducing substance, which disappeared on oxidase treatment, gave the value for "true" ascorbic acid.

The treatment with the oxidase in each case was carried out in the following way. The p_H of the solution (usually 10 c.c.) was brought to 5.6 by the addition of a drop or two of NaOH. 2 C.c. of *M*-acetate buffer (p_H 5.6) and 3 c.c. of the enzyme solution (whose potency had been previously determined with pure ascorbic acid)* were added and the contents incubated at 40° for 30 minutes. The solution was made up to a definite volume and titrated against the dye. The difference in the dye value before and after incubation with the oxidase gave the measure of "true" ascorbic acid. The results are given in Table I, which shows that with these foodstuffs hot H_2S treatment gave a higher value for "true" ascorbic acid than by simple trichloroacetic acid treatment or by cold H_2S treatment.

* It is highly important that the amount of oxidase used should be more than sufficient to oxidise all the ascorbic acid likely to be present in the tissue extract. 3 C.c. of the present oxidase preparation were capable of oxidising 0.4 mg. of ascorbic acid under our conditions of experiment. The aliquots of the tissue extracts were, therefore, so chosen that they contained 0.3 mg. or less of reducing substance.

TABLE I.

(Mg. of ascorbic acid per 10 g. of foodstuff.)

Expt. No.	Foodstuff investi- gated.	Total reducing substances initially present			Reducing substances remaining after oxidase treatment			"Total, true" ascorbic acid		
		(a).	(b).	(c).	(a)	(b).	(c).	(a).	(b).	(c).
1	Cabbage	1'060	4 952	6 630	0 340	2'280	2'540	0 720	2'670	4'090
2	Cabbage	2'080	4 330	5 200	0'600	1'830	2 540	1'480	2 500	2'660
3	Patol (<i>Trichosan- thes dioica</i>)	1'130	2'476	2'971	0 060	1'200	1 300	1'130	1 276	1'671
4	Patol	1'223	3'058	3'466	0'000	1'240	1'300	1'223	1 818	2'166
5	Onion	1'040	1'543	1'654	0'000	0'000	0'000	1'040	1'543	1'654
6	Onion	0 945	1'300	1'567	0'307	0'594	0'940	0'638	0'706	0'726

(a) Simple trichloroacetic acid extraction.

(b) H_2S treatment in cold condition.(c) H_2S treatment in hot condition.

*The Application of the Present Method to the Estimation of
"total, true" Ascorbic Acid in Foodstuffs.*

Since the method given under (c) above appears to give higher values for true ascorbic acid than the ordinary procedure, it has been applied as a routine method of estimation to a number of other foodstuffs. The values obtained by simple trichloroacetic acid treatment in the ordinary way and also after oxidase treatment are given for comparison. The results are shown in Table II.

Stability of the Oxidase prepared from Cucumber.

As the method described above under (c) would require ascorbic acid oxidase as a regular reagent of known potency, it was considered desirable to investigate the stability of the oxidase under the following conditions of storage.

(1) The dry oxidase preparation was preserved in a vacuum desiccator at room temperature (27-30°) and also at 8-10°.

TABLE II.

No.	Bengali name.	English name.	Botanical name.	Mg. of ascorbic acid per 100 g. of foodstuffs.	Trichloroacetic acid extraction and oxidase treatment.	Hot H ₂ S and oxidase treatment. (present method).
1	Kancha lanka	Green chillies	<i>Capiscus indicus</i>	50.0	50.0	68.9
2	Fulkopi	Cauliflower	<i>Brassica oleracea</i>	50.0	50.0	62.6
3	Begoon	Brinjal	<i>Solanum melongena</i>	4.7	4.7	5.7
4	Mula	Radish	<i>Raphanus sativus</i>	20.8	20.8	23.4
5	—	Soya bean		1.0	1.0	1.1
6	Anarash	Pine apple	<i>Ananas sativa</i>	69.3	69.3	73.7
7	Pepe	Papaya	<i>Carica papaya</i>	65.0	65.0	75.2
8	Kala	Plantain (ripe)	<i>Musa sapientum</i>	4.6	4.6	12.1
9	Kala	" (green)		9.5	9.5	14.5
10	Dalim	Pomegranate	<i>Punica granatum</i>	50.0	49.1	52.2
11	Bel	Wood apple	<i>Aegle marmelos</i>	86.7	60.4	61.9
12	Palang saak	Spinach	<i>Spinach oleracea</i>	14.9	14.9	52.0
13	Pun shak	—	<i>Bassella cardifolia</i>	41.6	41.6	89.3
14	Kalmi shak	Ipomoea	<i>Ipomoea reptans</i>	26.0	13.6	30.9
15	Matarshuti	Peas	<i>Pisum sativum</i>	19.8	19.8	23.1
16	Tomato	Tomato	<i>Lycopersicon esculentum</i>	27.3	27.3	39.6
17	Shalgom	Turnip	<i>Brassica campestris</i>	22.1	22.1	31.8
18	Chalkumro	White gourd	Var.			
19	Sasha	Cucumber	<i>Benincasacutapa</i>	9.5	9.5	22.2
20	Batapji lebu	Shaddock	<i>Cucumis sativus</i>	9.5	9.5	14.9
21	Apel	Apple	<i>Citrus decumana</i>	26.0	26.0	27.7
22	Am	Mango	<i>Pyrus nolas</i>	4.6	4.6	4.8
23	Angur	Grapes	<i>Mangifera indica</i>	6.5	6.5	12.0
24	Mocha	—	<i>Vitis vitifera</i>	5.5	2.4	3.6
25	Shim	Bean	—	115.6	115.6	149.7
26	Kul (Narkel)	—	—	7.0	2.3	19.5
27	Kumra	Pumpkin	—	46.2	46.2	62.7
28	Shakalu	—	—	3.0	2.5	6.5
29	Kamla lebu	Orange	<i>Citrus aurantium</i>	20.8	20.8	24.7
30	Lebu	Lemon	<i>Citrus medica</i>	30.6	30.6	31.4
				36.1	36.1	36.1

(2) The dry preparation was stored in ordinary colourless and amber coloured stoppered bottles at both room temperature and 8-10°.

(3) Aqueous solutions of the enzyme of definite concentrations were preserved with a drop or two of toluene in colourless and amber-coloured stoppered bottles at 8-10°.

The activity of the enzyme was investigated initially and after intervals of 2, 7 and 15 days. The results are given in Table III.

TABLE III.

The dry enzyme preparation (0.05 g.) was dissolved in 16 c. c. of water and 3 c.c. of the enzyme solution were employed for the estimation of this activity.

State of material.	Conditions of storage.	Activity of the enzyme solutions (3 c.c.) measured in terms of ascorbic acid (mg.) oxidised under specified condition.			
		Initial.	After 2 days.	After 7 days.	After 15 days.
Dry powder	Vacuum desiccator at 8-10°	(1) 0.4	0.4	0.3	0.2
		(2) 0.6	0.5	0.4	0.2
	Vacuum desiccator at room temperature (27-30°)	(1) 0.4	0.4	0.3	0.2
		(2) 0.6	0.5	0.4	0.2
	Colourless bottle at 8-10°	(1) 0.4	0.4	0.3	0.2
		(2) 0.6	0.5	0.4	—
"	Colourless bottle at room temperature (27-30°)	(1) 0.4	0.4	0.3	0.1
		(2) 0.6	0.5	0.4	0.1
	Amber-coloured bottle at room temperature (27-30°)	(1) 0.4	0.4	0.3	0.1
		(2) 0.6	0.5	0.4	0.1
Aqueous solution	Colourless bottle at 8-10°	(1) 0.4	0.4	0.2	0.1
		(2) 0.6	0.5	0.3	0.1
	Amber-coloured bottle at 8-10°	(1) 0.4	0.4	0.2	0.1
		(2) 0.6	0.5	0.3	0.1

DISCUSSION

The previous method (Sen-Gupta and Guha, 1937, *loc. cit.*) for the estimation of total ascorbic acid in foodstuffs has been modified. The method now consists in heating an aqueous suspension of the foodstuff in H₂S and in titrating (after removal of H₂S by CO₂ or N₂) the solution, before and after treatment with ascorbic acid oxidase of known potency,

against 2:6-dichlorophenol-indophenol. The method is described under (c). It appears to give the value for "total, true" ascorbic acid comprising free ascorbic acid, dehydroascorbic acid and ascorbigen, if any. The method has been applied to a number of foodstuffs and the results show that in most cases the values obtained are appreciably higher than those obtained by the usual method of trichloroacetic acid extraction. Biological experiments to check these results are in progress.

As ascorbic acid oxidase preparation would be a routine reagent in this method, its stability has been investigated under different conditions of storage. Colourless and coloured bottles do not seem to make any difference. The enzyme progressively deteriorates both in the dry state and in aqueous solution, but more rapidly in the latter state. A dry preparation of the enzyme is recommended to be used for periods not exceeding 7 days and aqueous solutions for periods not exceeding 3 days.

Our thanks are due to the Indian Research Fund Association for financing this research.

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Received September 14, 1939.

A NOTE ON THE ACTION OF NITROSYL CHLORIDE ON MONOBROMOMALONAMIDES.

By M. P. SHAH AND V. B. THOSAR.

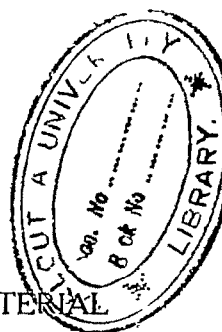
The action of nitrosyl chloride on substituted malonamides, $\text{CH}_2(\text{CONHR})_2$, leads to $\text{HO}\cdot\text{N}:\text{C}(\text{CONHR})_2$ which give characteristic alkali and iron salts (Whitely, *J. Chem. Soc.*, 1903, **83**, 24). Bromomalonamides of the type $\text{CHBr}(\text{CONHR})_2$ were expected to yield nitroso compounds, $\text{C}(\text{NO})\text{Br}(\text{CONHR})_2$ by the action of nitrosyl chloride. Contrary to these expectations compounds of the type $\text{ClBrC}(\text{CONHR})_2$ were obtained. The same compounds are obtained by the action of the usual chlorinating agent, sulphuryl chloride, on monobromomalonamides. That nitrosyl chloride may behave in this manner is not unknown (Solonia, *J. Russ. Phys. Chem. Soc.*, 1898, **30**, 431).

Reaction of Nitrosyl Chloride and Monobromomalon-di-p-toluidide.—The amide (5 g.) suspended in benzene (150 c.c.) was saturated with dry gaseous nitrosyl chloride at 0° . At the end of one hour the major portion of the amide went into solution which assumed a dark red colour. The mixture was then heated under reflux till the condensed vapour became colourless. The benzene solution was then filtered hot and concentrated. The substance, thus obtained, was collected, washed with light petroleum and crystallised from alcohol, m.p. 135° . (Found: N, 7.04; Halogen, 29.11. $\text{C}_{27}\text{H}_{18}\text{O}_2\text{N}_2\text{ClBr}$ requires N, 7.08; Halogen, 29.2 per cent).

Reaction of Nitrosyl Chloride and Monobromomalon-dibenzylamide.—The reaction was carried out as above. The product after recrystallisation from alcohol melted at 153° . (Found: N, 7.32; Halogen, 29.2. $\text{C}_{17}\text{H}_{16}\text{O}_2\text{N}_2\text{ClBr}$ requires N, 7.08; Halogen, 29.2 per cent).

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Received October 6, 1939.



NITROGEN FIXATION IN SOIL NOT WHOLLY A BACTERIAL PROCESS.

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Fixation of nitrogen may not be wholly associated with bacterial activity as has hitherto been believed but can also take place under completely sterile conditions even with silica, zinc oxide, aluminium oxide, ferric oxide, etc. when they are mixed with energy materials such as glucose and others. The energy available from the oxidation is probably responsible for such fixation in the complete absence of micro-organisms, and the reaction in light is much greater than in the dark.

In recent communication it has been shown that nitrogen fixation in the soil on the addition of energy materials is very much greater in light than in the dark, although the azotobacter and total bacterial numbers are greater in dark than in light. We have concluded from our experiments that nitrogen fixation in soils, which has hitherto been considered to be entirely a bacterial process, is markedly accelerated by light absorption and does not depend on bacteria alone.

In order to clear up the mechanism of nitrogen fixation, experiments have been carried on with surfaces other than soil and under completely sterile conditions and the results obtained are recorded in this paper.

EXPERIMENTAL.

Experiments with Oxide of Metals.

50 G. of silica, zinc oxide, ferric oxide and aluminium oxide were mixed separately with one gram of glucose and 25 c.c. of water in enamelled basins (16 cm. in diameter). One set of the basins was exposed to sunlight and the other corresponding set, covered with black cloth to exclude light, was placed side by side along with the exposed ones. Whenever the oxides were dried up 20 c.c. of water were added. Control experiments were also carried on. To start with all the oxides were analysed for their total nitrogen content and after the end of the exposure both the exposed and covered oxides were analysed. The total bacterial numbers in both the cases were also determined. In the following two cases, the zinc oxide and silica used were pure and free from nitrogen. Experiments were started on September 12, 1938 and analysed on November 1, 1938 after exposure,

The following results were obtained.

Substance.	Sunlight.		Dark.	
	Total nitrogen.	Total carbon.	Total nitrogen.	Total carbon.
Silica	0.0172%	0.2946%	0.0054%	0.5928%
Zinc oxide	0.0152	0.0268	0.0054	0.5312

More experiments were started on December 9, 1938 and the materials analysed on January 10, 1939.

Substance.	Exposed.			Dark.		
	Nitrogen		Total carbon.	* Total bacteria	Total N.	Total C.
	Original	Total				* Total bacteria.
Zinc oxide	0 %	0.011 %	0.0268 %	0.16	0.0038%	0.6172 %
Aluminium oxide	0	0.0084	0.4150	0.46	0.0044	0.5824
Silica	0.016	0.028	0.3304	1.08	0.0212	0.5088
Ferric oxide	0.024	0.0336	0.4150	0.92	0.028	0.5624

* In millions per gram of oxide.

Controls analysed on January 11, 1939.

Substance.	Exposed.	Dark.
Zinc oxide	Nil	Nil
Aluminium oxide	"	"
Silica	0.0136	0.0144
Ferric oxide	0.0208	0.0224

The above results indicate that just as with soil, nitrogen fixation is possible with surfaces like the metallic oxides used which behave as active surfaces. In these cases also the nitrogen fixation is always greater in the exposed oxides than in the covered ones although the total bacterial numbers are greater in the latter than in the former.

Experiments under Sterile Conditions.

50 G. of soil were weighed into sterile quartz flasks of 500 c.c. capacity and 40 c.c. of sterile distilled water were added. They were plugged with cotton wool and then sterilised at 20 lbs. pressure for 4 hours. After the flasks were cooled 1 g. of each of the carbohydrates used was added to each of the flasks and again sterilised for 1 hour. After the sterilisation one set of the flask was exposed to sunlight and the other corresponding set was covered with black cloth to exclude light and placed side by side along with the exposed flasks. In the same way some experiments were carried in pyrex glass flasks.

In the case of silica and zinc oxide, the materials and 40 c.c. of sterile distilled water were added to the sterilised quartz flasks and sterilised for 1 hour at 20 lb. pressure. Then 1 g. of glucose was added and again sterilised for half an hour at 15 lb. pressure. In all the above experiments controls were kept. At the end of the exposure the materials present in both the exposed and covered vessels analysed simultaneously. Before analysis all the soils were tested for bacterial contamination and found to be perfectly sterile.

In the case of sterile soils the experiments were started on September 12, 1938. Soils in quartz flasks were analysed on March 1, 1939.

Analysis of the Original Soil.

Substance	Sunlight.		Dark.	
	Total N.	Total C	Total N	Total C.
Inulin	0.0464 %	0.7732 %	0.0424 %	0.9924 %
Arabinose	0.0448	0.7904	0.0424	0.9826
Fructose	0.0464	0.7635	0.0432	0.9786
Lactose	0.0456	0.7816	0.0424	0.9924
Glucose	0.0456	0.7635	0.0432	0.9562
Mannitol	0.0456	0.7732	0.0424	0.9826
Glycerol	0.0448	0.8448	0.0424	0.8968
Galactose	0.0456	0.7732	0.0424	1.0056
Maltose	0.0464	0.7735	0.0424	1.1026
Dextrin	0.0464	0.7732	0.0432	0.9826
Starch	0.0448	0.9264	0.0424	1.1206
Control	0.0408	0.3986	0.0416	0.4208

Soils in pyrex flasks were analysed on March 27, 1939.

Substance	Exposed.		Dark.	
	Total N.	Total C.	Total N	Total C.
Inulin	0.0456 %	0.8968 %	0.0424 %	1.1264 %
Arabinose	0.0448	0.9086	0.0424	1.0086
Fructose	0.0448	0.9156	0.0432	0.9924
Glucose	0.0448	0.8892	0.0424	0.9638
Starch	0.0432	1.1026	0.0424	1.1284
Control	0.0416	0.4208	0.0416	0.4208

Experiments with sterile silica and zinc oxide were started on January 6, 1939.

Substance.	Exposed.		Dark.	
	Analysed on May 6, 1939.		Analysed on June 13, 1939	
	Total nitrogen	Total carbon	Total nitrogen	Total carbon.
Silica	0.0082%	0.49/2%	0.0038%	0.6172%
Zinc oxide	0.0060	0.3882	0.0038	0.5892

In the controls no nitrogen fixation was observed

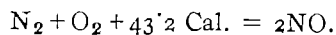
From the results recorded above it is clear that appreciable fixation of nitrogen takes place when energy materials and sterile soil are exposed to light under perfectly sterile conditions.

It may be argued that the fixation of nitrogen can also take place in sterile soil because of the enzymes left when the soil is sterilised. But our results showing appreciable amounts of nitrogen fixation with silica and zinc oxide under completely sterile conditions dispose of the enzyme view, because no bacteria are associated with pure chemicals like silica and zinc oxide. Moreover, if nitrogen fixation were to be an enzymatic reaction, there should have been the same amount of fixation in the dark as in light, but it is not so. Again, we have to remember the fact that under the conditions the soils were sterilised, the enzymes, if any, will surely lose their activity.

It is well known that there is increase of nitrogen in a system containing a culture of pure azotobacter suspended in a medium containing calcium carbonate, small quantities of iron salts and a solution of energy material. In this case also, along with the marked increase in the number of azotobacter, a chemical reaction causing a fixation of nitrogen may take place on the surface of the calcium carbonate particles. The bacteria present in the system act as converters of energy, and that is why they help in bringing about the glucose oxidation



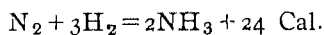
A part of the energy of this chemical change is actually utilised in formation of bacterial cells whilst another part is utilised in effecting the combination of nitrogen and oxygen according to the equation



There are two schools of thought regarding the mechanism of nitrogen fixation. Winogradsky and others believe that ammonium salt is the first product to be formed. As a matter of fact in culture experiments there is an increase of ammonium salt in the system in the first few days of the experiment. Moreover, in all our experiments with soils in dishes or in

fields we have observed an increase of ammonium salts within a week after the addition of energy materials like carbohydrates, glycerol, molasses, etc. There is no doubt, therefore, that ammonium salts are readily formed or they increase in the process of nitrogen fixation.

It is quite possible that hydrogen and nitrogen slowly combine on the soil surface according to the equation

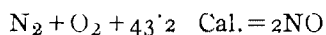


as in the Haber-Bosch process. There is also another way of explaining the formation of ammonium salts. The reaction involving the oxidation of energy materials liberates large amounts of energy, which is very much greater than the energy required for the decomposition of water



The atomic hydrogen formed in this reaction may readily combine with nitrogen on the surface of soil or zinc oxide or any other surface forming ammonium salts.

Another view point is that on the soil surface there is an intimate contact of nitrogen and oxygen gases and they may combine utilising the energy of the carbohydrate oxidation



As most of our experiments have been carried on under completely aerobic conditions, this mechanism may be a possible one. When the oxide of nitrogen is once formed nitrate may be easily obtained. The nitrate in its turn is readily reduced to ammonium salt in presence of carbonaceous substances. It is of considerable importance to note here that Dhar and Mukerji (*J. Indian Chem. Soc.*, 1934, 11, 727) discovered the formation of small amounts of amino-acids and large amounts of ammonium salts by exposing the solutions of nitrate like glucose, canesugar in presence titania as a photocatalyst and exposed to sunlight or artificial light. It is quite possible, therefore, that the nitrate, which may be produced, may lead to the formation of ammonium salts and amino-acids to be partially used up by the bacteria. Hence the presence of ammonium salts in nitrogen fixation and increase of total nitrogen is explained.

We have been able to establish in these laboratories that all energy materials can be converted into carbon dioxide and water by passing air through their solutions or suspensions when exposed to sunlight or artificial light or mixed with surface like ferrous hydroxide, manganous hydroxide, silica, etc. It appears, therefore, that in the oxidation of energy materials

at the ordinary temperature, living matter need not be associated and this transformation of energy materials causes nitrogen fixation without the presence of micro-organisms. These results seem to have an important bearing on our conceptions of bacterial processes.

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C O N C L U S I O N.

When glucose is mixed with silica, zinc oxide, aluminium oxide, ferric oxide etc. and exposed to air and light, there is appreciable nitrogen fixation. The nitrogen fixation in light is greater than in the dark under comparable conditions.

When energy materials like inulin, arabinose, fructose, lactose, glucose, mannitol, glycerol, galactose, maltose, dextrin and starch are mixed with sterile soil in either quartz or pyrex glass vessels there is appreciable nitrogen fixation even under completely sterile conditions, which is greater in light than in dark and in quartz than in glass vessels under identical conditions.

With glucose mixed with silica or zinc oxide under completely sterile conditions and exposed to air and light, there is also nitrogen fixation both in light and dark, it being greater in the former condition than in the latter.

From the foregoing results it is inferred that nitrogen fixation can take place under completely sterile conditions and may not be associated with bacterial activity as has hitherto been believed and that the energy available from the oxidation of glucose or other energy materials can lead to nitrogen fixation even in the complete absence of micro-organisms.

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Received August 18, 1939

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ELECTROCHEMICAL PROPERTIES OF STEARIC ACID HYDROSOL. PART I.

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For reproducible p_H measurements, coating the platinum electrode with a thin black deposit and then washing with water in a current of hydrogen has been found satisfactory. Electrometric titration of the sols with $Ba(OH)_2$ and $Ca(OH)_2$ show that they should be regarded as a two phase system. The salt formed by the interaction forms a separate phase. The NaOH titration curves resemble to some extent those of weak acid with a strong base. The maximum buffer index, however, does not correspond to half neutralisation but are shifted towards the right corresponding to the addition of larger amounts of alkali. The p_H remains constant between 6.5 and 7.0 in the $Ba(OH)_2$ titrations and between 9 and 9.5 in the NaOH titrations. The total acidity as observed from the inflexion points in the curves agrees fairly with the stoichiometric concentration of the acid. Titration in presence of neutral salts gives the same total acidity as that of the pure sol. Ultrafiltrates of the sol and salt mixtures give only a fraction of the total acidity of the sol.

In a previous paper one of us (S. Mukherjee, *J. Indian Chem. Soc.*, 1937, **14**, 17) has shown that the titration curves of palmitic acid hydrosols with different bases give different total acidities and that their properties could be best understood if they were considered to be heterogeneous systems. Kawamura (*J. Phys. Chem.*, 1926, **30**, 1364), while engaged in investigations on the adsorption of $Ba(OH)_2$ and NaOH by solid stearic acid, found that stearates are formed by the interaction and that during the formation the p_H remains constant at 8.0 in the $Ba(OH)_2$ and at 10.0 in the NaOH titrations. In our sols, however, as will be shown later on the p_H remains constant between 6.5 and 7.0 in the $Ba(OH)_2$ and between 9 and 9.5 in the NaOH titration.

Iyer (*Half-yearly J. Mysore Univ.*, 1932, 1) has previously observed different total acidities of stearic acid hydrosols in titrations with NaOH and $Ba(OH)_2$. The form of the titration curves were also found to be different for the two bases. While it appears from the investigations of Iyer (*loc. cit.*) that there is no definite stoichiometric relationship between the amount of alkali used and the acid which reacts with it, S. Mukherjee (*loc. cit.*) using palmitic acid sols observed a definite stoichiometric relationship in titrations with baryta. It was thought desirable to undertake a fuller investigation of stearic acid sols. The results obtained in this work are of some interest in relation to the properties of colloidal acids, e.g.

hydrogen clays which also show a difference in the total acidities when titrated with different bases (*Indian J. Agric. Sci.*, 1936, **6**, 517, 555).

EXPERIMENTAL.

Merck's pure stearic acid and Merck's absolute alcohol were used. The sols were stocked in the same manner as described for palmitic acid sols (S. Mukherjee, *loc. cit.*). The p_H of the sol was mostly measured with the hydrogen electrode against normal calomel electrode (E.M.F. 0.2823 volt at 35°) but the glass electrode and the quinhydrone electrodes were also employed for comparison. A Leeds Northrup K-type potentiometer in conjunction with a Hartmann and Braun galvanometer was used for measuring the potentials. p_H measurements with the glass electrode were carried out with the Morton System of electrodes and an electrometer valve potentiometer obtained from the Cambridge Instrument Company. Before each measurement the glass electrode was checked with two buffer solutions differing by one unit of p_H . To avoid contamination the potentiometric titrations were carried out in a pyrex glass titration cell provided with airtight ground glass joints. The experimental arrangements in other regards were the same as described in the previous paper. A perforated porcelain bed and 'cella' membranes were employed for ultrafiltration. A water thermostat was used at 35° + 0.05°.

The measurement of the p_H of poorly buffered solutions having low hydrogen ion concentrations is attended with various difficulties. Extreme care is necessary to prevent the access of traces of impurities. The hydrogen electrode is itself often a source of contamination (Kolthoff and Kameda, *J. Amer. Chem. Soc.*, 1929, **51**, 2888). A freshly platinised electrode, which has been washed as usual, often shows a lowering of the p_H as the passage of hydrogen continues, presumably from the liberation of acids adsorbed during platinisation. The use of thinly platinised electrodes with bright deposit recommended by Kolthoff and Kameda (*loc. cit.*) has the disadvantage that the catalytic activity of the electrodes for the reaction, $H^+ + e = H$, dies away within a short time. In previous work in this laboratory erratic variations in the p_H values of such colloidal solutions have often been observed with the hydrogen electrode. On washing and replatinisation its behaviour improved and reproducible values were obtained. Since stearic acid sols have very low hydrogen ion concentrations of the order of $10^{-6}N$, and specific conductivities of the order 10^{-6} mho, the following precautions were taken. Every day, after the measurements,

the platinum deposit on the electrode was removed and the latter was replatinised with a thin dark grey deposit. After washing thoroughly the electrode was kept immersed in conductivity water for about half an hour in a current of hydrogen. They were then again washed with conductivity water and used for the measurements. This procedure proved satisfactory.

The p_H values of stearic acid sols are given in Table I, (g) indicates the glass electrode. Altogether some twelve to sixteen separate p_H determinations were made on each of the sols on different dates, of which only four or five are given below. The rest showed similar variations.

TABLE I.

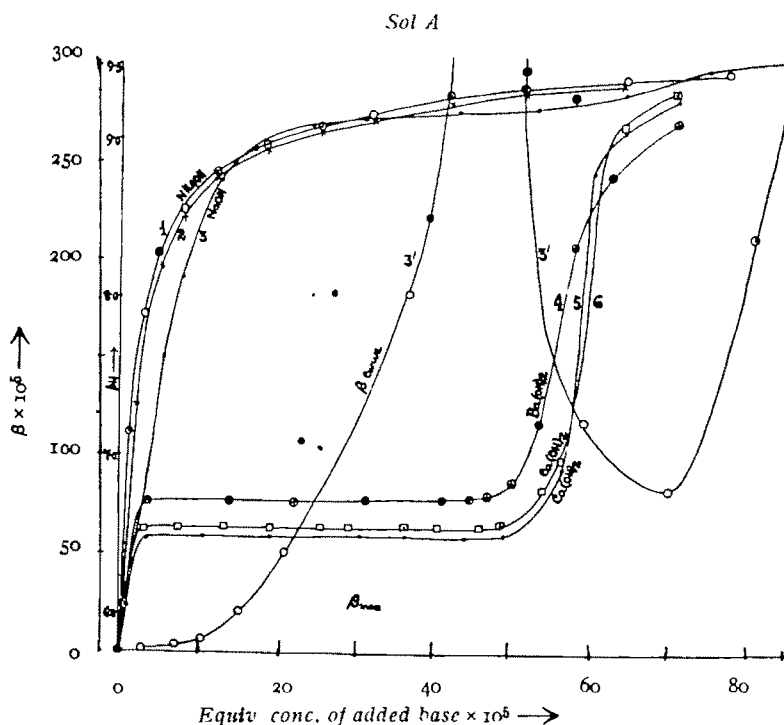
Sol A		Sol B		Sol C	
Date.	p_H .	Date.	p_H .	Date.	p_H .
5-1-37	5'33	4-2-37	5'45	2-3-37	5'36
14-1-37	5'40	10-2-37	5'39(g)	8-3-37	5'32
25-1-37	5'37	14-2-37	5'32	19-3-37	5'32
		16-2-37	5'33	23-3-37	5'37
29-1-37	5'41	23-2-37	5'36	27-3-37	5'40
4-2-37	5'41	25-2-37	5'39		Mean 5'35
	Mean 5'384		Mean 5'36		

It will be seen, that with the precautions taken the reproducibility of the p_H values is fairly satisfactory. The best reproducibility is observed with sols B and C. A cause of the variation perhaps lies in the tendency of the sol to form a deposit of stearic acid on surfaces with which it comes in contact. At the end of the measurement a thin grey coating was observed on the platinum surface and a coating was also noticed on the surface of the glass electrode. The quinhydrone electrode does not give a steady value of the E.M.F. of this sol.

Titration Curves.

The titrations were often repeated to check their reproducibility. The agreement among the replicated curves is satisfactory (see for example curves

FIG. 1.

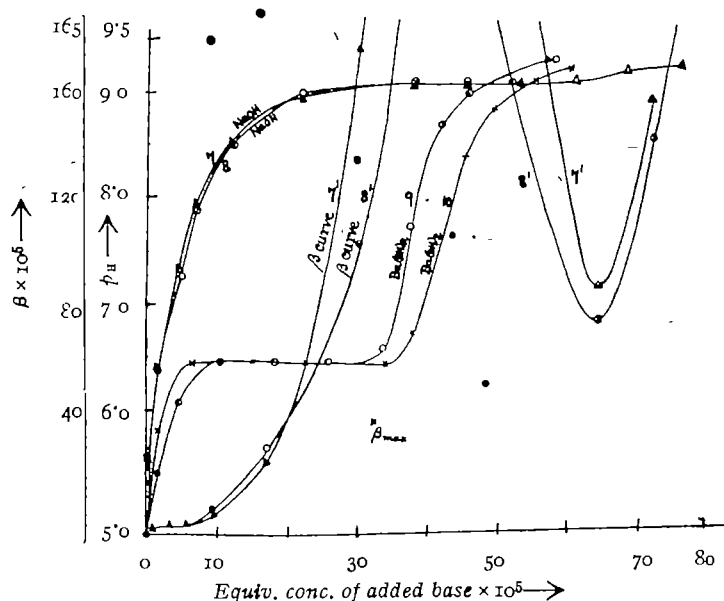


1 and 2, 5 and 6 for sol A and 7 and 8 for sol B, curves 17 and 18 for sol D). In some cases, however, the agreement is not so close, *e.g.*, curves 9 and 10 for sol B. Irregularities in the kinetics of the interactions appear to be responsible for these discrepancies. The time allowed for the reaction and the rate of stirring have considerable influence on the course of such reactions. This is evident from the titration curve (Fig. 4, curve 19). In this titration increasing amounts of alkali were added to a known volume of the sol in a series of Jena glass bottles. After vigorously shaking the contents, they were kept overnight and the p_H was determined next day. Further complications arise where the sol coagulates, *e.g.*, during titrations with $Ba(OH)_2$ or with neutral salts. Though an attempt has been made to keep the conditions uniform it is not possible to avoid slight variations.

The total acidities have been calculated from the inflexion points with $Ca(OH)_2$ and $Ba(OH)_2$, the inflexion point can be located with an accuracy of about $\pm 2.5\%$ of the total acidity. With $NaOH$, the slope is not so well expressed and the total acidities are liable to larger uncertainties, as much as $\pm 7\%$.

FIG. 2.

Sol B.



On gradually adding NH_4OH (Fig. 1, curves 1 and 2) the p_H first rises steeply. The slope of the curve decreases but no inflexion point is observed. The reaction is weak till a p_H near about 9 is reached when the particles gradually dissolve. NaOH (Fig. 1 curve 3, Fig. 2 curves 7 and 8); resembles NH_4OH to some extent in its interaction but after a flat portion between p_H 9.0 and 9.2, the titration curves rise again and show a weak though distinct inflexion near about p_H 9.3 resembling that of a weak acid with a strong base.

The p_H values at half neutralisation diminishes with an increase in the stearic acid constant (1.77×10^{-6} at 35° , *vide* Part II) and concentration (*vide infra*) similar to that of the stearic acid, the p_H at half neutralisation at the following concentrations are given below.

Conc. $\times 10^5 \text{N.}$	p_H at half neutralisation.
60	5.77
30	5.78

but the variations observed here are larger than those calculated and the half neutralisation point occurs at a much higher p_H .

Other differences are brought out by a calculation of the buffer indices (Van Slyke, *J. Biol. Chem.*, 1922, **22**, 525). For a weak acid in true solution Van Slyke's expression for the buffer index β is as follows

$$\beta = \frac{db}{dp_H} = 2.302 \frac{a.k.[H^+]}{k + [H^+]^2} + [H^+] + [OH^-]$$

where b is the amount of base added, a , the initial concentration of the acid. The other symbols have their usual significance.

At the point of half neutralisation β reaches a maximum value, $\beta_{\max} = 0.575a$, which should be independent of the dissociation constant of the acid.

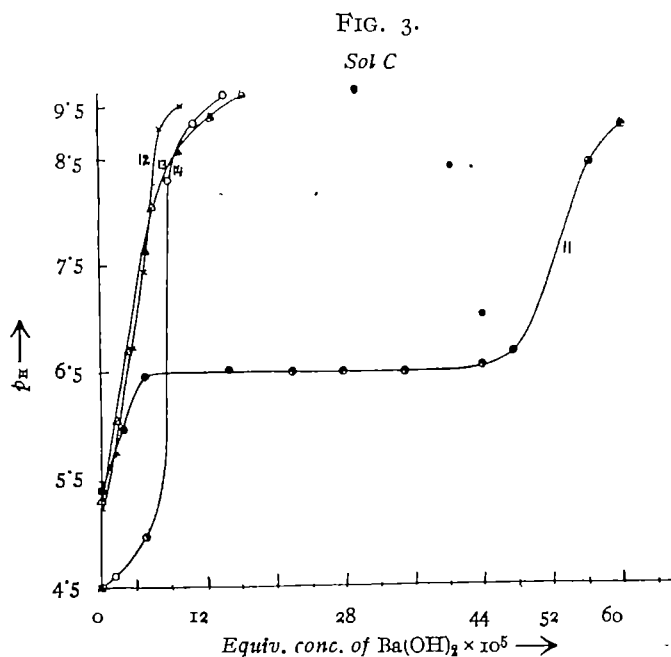
From the NaOH titration curves of sols A and B, the buffer indices have been calculated and plotted against the concentration of the added base in Fig. 1, (curve 3') and Fig. 2 (curves 7' and 8') respectively. The maximum buffer index has unusually large values. The maxima do not correspond exactly to half-neutralisation but are shifted towards the right corresponding to the addition of a larger amount of the alkali. These titration curves thus differ essentially from that of a homogeneous solution of a weak acid.

The titration curves obtained with $Ba(OH)_2$ (Fig. 1, curve 4; Fig. 2, curves 9 and 10; Fig. 3, curve 11) and $Ca(OH)_2$ (Fig. 1, curves 5 and 6) resembles each other closely. A strong buffering is indicated between p_H 6.5 and 7.0. The form of the curves is similar to those observed by S. Mukherjee (*loc. cit.*) with palmitic acid sols and the same bases. The p_H values at the inflexion points (*vide* Table II) are, however, somewhat higher for the stearic acid sols. Sol D was prepared from stearic acid obtained by twice crystallising Merck's pure stearic acid from absolute alcohol and is considered to be free from certain impurities present in sols A, B, C.

TABLE II.

Sol.	Curve No.	Base used.	p_H in the horizontal part.	p_H at inflexion
A	4	$Ba(OH)_2$	6.70	7.66
	5	$Ca(OH)_2$	6.47	7.76
	6	"	6.53	7.30
B	9	$Ba(OH)_2$	6.48	7.30
	10	"	6.48	7.50
C	11	$Ba(OH)_2$	6.50	7.75
D	16	$Ba(OH)_2$	6.90	8.50
	17	"	6.95	8.50
	18	"	6.7	8.50

A difference in the titration curves with NaOH and $\text{Ba}(\text{OH})_2$ has been observed by Iyer (*loc. cit.*). With NaOH he obtained a total acidity two-thirds of that with baryta and concluded that an acid sodium stearate, $2\text{NaA} \cdot \text{HA}$ and a normal barium salt were formed. The results given below show that in both cases neutral salts are formed.



The total acidities obtained from the titration curves and the stoichiometric concentration of stearic acid in the sols, as determined by extraction with ether are given in Table III for comparison.

TABLE III.

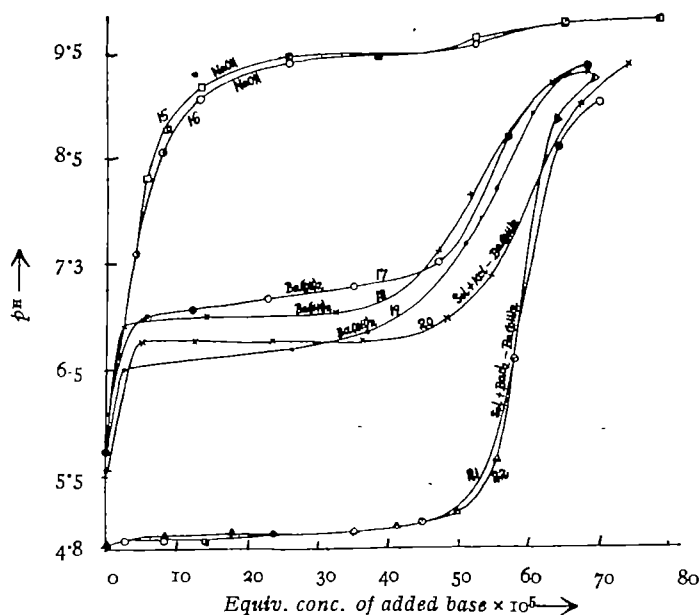
Sol.	Base used.	Curve No.	Total acidity by titration.	($N \times 10^5$) by extraction with ether.
A	$\text{Ca}(\text{OH})_2$	6	59.5	
	NaOH	3	60.0	
B	$\text{Ba}(\text{OH})_2$	10	41.3*	
	NaOH	7	65.0	
	"	8	65.0	
C	$\text{Ba}(\text{OH})_2$	11	52.0	53.1
D	$\text{Ba}(\text{OH})_2$	18	59.0	
	$\text{Ba}(\text{OH})_2$	16, 17	58.0	58.8
	NaOH	15	58.5	59.2

* This unusually low value is due to inefficient stirring, some of the unreacted stearic acid molecules escaping reaction being enveloped over by insoluble barium stearate formed.

The titration curves can be understood, if one considers the heterogeneous nature of the system. The colloidal particles of stearic acid constitute the solid phase and the intermicellar liquid contains its saturated solution (and perhaps some impurities in traces in the sols A, B, C). When neutralised with $\text{Ca}(\text{OH})_2$ or $\text{Ba}(\text{OH})_2$, the Ca or Ba salts of stearic acid, which are insoluble in water, are expected to form a new solid phase. The p_H should at first rise till the solubility product of the salt is reached. Thereafter so long as the two solid phases namely the solid acid and the solid salt co-exist, the p_H should remain unchanged. On the addition of further and sufficient amounts of the base, the solid particles of the acid will disappear and the p_H should again rise sharply and a point of inflexion should mark the neutralisation point. These features are borne out by the titration curves.

FIG. 4.

Sol D.



With NaOH and NH_4OH , the resulting salts are soluble but are hydrolysed. The p_H should sharply rise as happens with a weak acid and a strong alkali. On continued addition of the alkali, the interaction will be weak till a p_H is reached when stearate ions are stable in solution. At and beyond this stage the solid acid dissolves more and more and an inflexion should occur at the equivalence point. On account of the greater hydrolysis of the ammonium salt, the inflexion point of the corresponding

curve is rendered imperceptible. The unneutralised acid is mostly present in the solid form upto the neighbourhood of the equivalence point and the p_H should be higher throughout this portion than what it would be if the same amount of acid were in true solution. The p_H at half neutralisation should therefore vary with concentration and the buffer capacity curve should also have a different form than for a truly dissolved acid.

Neutralisable Acids in presence of Neutral Salts.

S. Mukherjee (*loc. cit.*) found that in presence of barium chloride the whole of the palmitic acid was not neutralised by $Ba(OH)_2$. The incomplete neutralisation was attributed to the inclusion of some of the fatty acid molecules in the coagulum formed by the addition of $BaCl_2$ which did not react with the base under the conditions of the experiment. This was confirmed in the preliminary experiments with stearic acid but it was not thought that it would be possible to ensure true equilibrium. Salt was added inside the cell and the contents were then vigorously stirred and titrated. The results show that the complete equilibrium was attained under these conditions. Sols D and E were made 0.1N in respect of KCl and $BaCl_2$ and then titrated with $Ba(OH)_2$ (Fig. 4, curves 20, 21, 22) and $NaOH$ (Fig. 5, curves 25, 26) respectively. The total acidities calculated from these curves are given below.

FIG. 5.
Sol E.

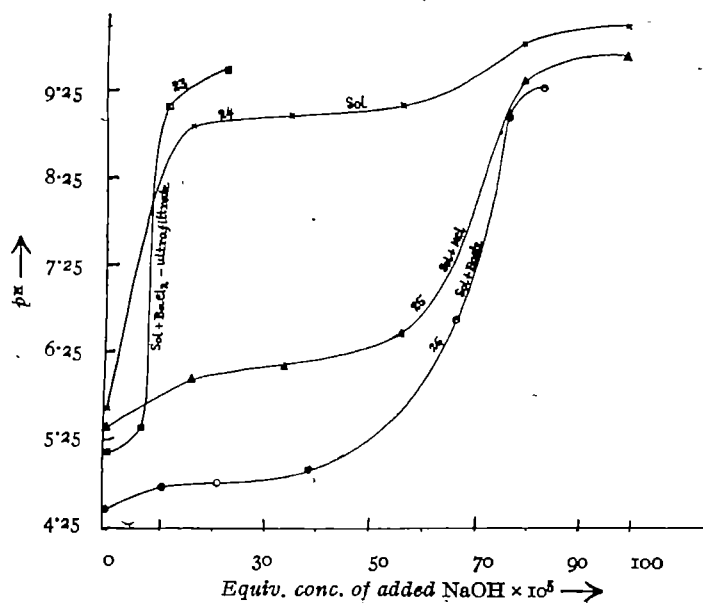


TABLE IV.

System	Base used	Total acidity $\times 10^5 N$	System	Base used	Total acidity $\times 10^5 N$
Sol D		59.0	Sol D + BaCl ₂	Ba(OH) ₂	58.7
Sol D + KCl	Ba(OH) ₂	60.0	Sol E	NaOH	74.9
Sol D + BaCl ₂	"	59.5	Sol E + KCl	"	73.9
			Sol E + BaCl ₂	"	75.0

The titration of the ultrafiltrate of the sol with Ba(OH)₂ show almost no total acidity (Fig. 3, curve 13). Ultrafiltrate of the sol-KCl mixture shows a total acidity of $5.6 \times 10^{-5} N$ (Fig. 3, curve 12). The ultrafiltrate of the BaCl₂ mixture has the typical properties of a strong acid and has a total acidity of $6.5 \times 10^{-5} N$. (Fig. 3, curve 14, Fig. 5, curve 23)

Our thanks are due to Prof. J. N. Mukherjee, D Sc. for his suggestions and kind interest in the work. We also take this opportunity to offer our thanks to the Calcutta University for affording laboratory facilities.

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Received September 9, 1939

ELECTROCHEMICAL PROPERTIES OF STEARIC ACID HYDROSOL. PART II.

•
BY N. P. DATTA.

The solubility of stearic acid at 35° and at 50° is $1.17 \times 10^{-5}N$ and $5.77 \times 10^{-5}N$ respectively. The dissociation constant of the acid has been found to be 1.7×10^{-6} at 35° and 2.6×10^{-6} at 50°. The p_H of the sol at 50° has been found to be less than that at 35° but the total acidity as is to be expected in the case in which the whole acid reacts, remains the same at 50°. The hydrogen ion activity of the sol increases on the addition of neutral salts. The solubility of barium and calcium stearates at 35° has been found to be $9.02 \times 10^{-6}N$ and $6.15 \times 10^{-6}N$ and at 50° $2.21 \times 10^{-6}N$ and $2.33 \times 10^{-6}N$ respectively.

In Part I of this series (*This issue* p. 563), electrometric titrations of stearic acid sols with bases NaOH, NH_4OH , $Ba(OH)_2$ and $Ca(OH)_2$ have been reported and it has been shown that the titration curves, though to some extent resemble those of a weak acid in true solution, are different and that the total acidity as obtained by titration agrees fairly well with the stoichiometric concentration of the acid as obtained by extraction. Neutralisable acids in presence of neutral salts like KCl, $BaCl_2$, $CaCl_2$ has been shown to be the same as that of the pure sol. An explanation has been offered from the phase rule considering the sol as a two phase system.

This paper deals with (i) the dissociation constant and solubility of the acid, (ii) the p_H change on the addition of neutral and hydrolysable salts and the solubility of calcium and barium stearates, and (iii) the effect of temperature on p_H , dissociation constant and solubility of the acid, nature of the titration curves with bases and neutral salts and the solubility of barium and calcium stearates.

EXPERIMENTAL.

The experimental arrangements were the same as described in Part I of this paper. Sols were prepared from stearic acid obtained by twice crystallising Merck's extra pure stearic acid from absolute alcohol and conductivity water. For conductivity determinations a cell of the Washburn type containing unplatinised electrodes was used. The electrodes were large and were very close to each other. The capacity of the cell was 50 c.c. and the cell constant 0.01. A 1000 cycle Vreeland oscillator was employed as the source of the alternating current and a Leeds and Northrup

roller conductivity bridge was used. A water thermostat was used at $35^{\circ} \pm 0.05^{\circ}$ and at $50 \pm 0.05^{\circ}$.

Dissociation Constant and Solubility of the Acid.

The titration curves of stearic acid sols with NaOH though to some extent resemble those of a weak acid with a strong base are different. The Henderson equation cannot therefore be applied to determine the dissociation constant of the acid from the potentiometric titration curves.

Taking colloidal stearic acid to behave as a weak acid obeying the law of mass action, and assuming the activity coefficient to be unity at these low concentrations, the following equation should hold:

$$[H^+][\text{Stearate}^-] = K[H\text{-Stearate}] = K.S_u = S$$

where S_u is the concentration of the undissociated acid in equilibrium with the solid phase. For the pure sol $[H^+] = [\text{Stearate}^-]$ and $[H^+]$ can be obtained from (i) the p_H of the sol, (ii) specific conductivity of the sol to which $[H^+]$ is related by the equation:

$$(u+v). [H^+] \times 10^3 = \rho \text{ (specific conductivity in rec. ohms).}$$

Further, in the case of the titration of the sol with NaOH, the above equation reduces to

$$[H^+][\text{Stearate}^-] = [H^+]\{[Na^+] + [H^+] - [OH^-]\} = K.S_u$$

since $[\text{Stearate}^-] = [Na^+] + [H^+] - [OH^-]$ and therefore, the $K.S_u$ can be evaluated from definite points in the titration curves, $[Na^+]$ being known from the amount of alkali added to the sol and $[H^+]$ and $[OH^-]$ from the p_H of the sol at that point. The results obtained by different methods are given in Table I.

TABLE I.

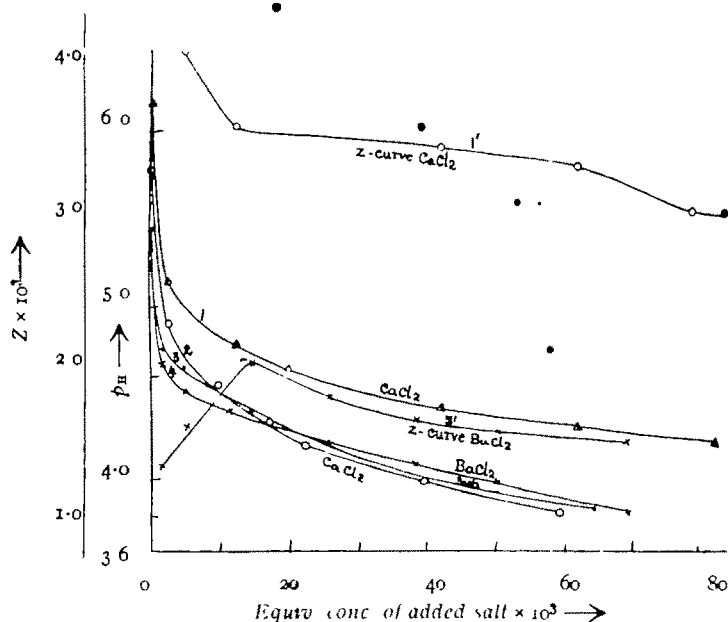
Sol	(a) p_H of sol.	(b) Sp. condty	$K.S_u \times 10^{-12}$			
			(c) Points in the curves.			
			$\frac{1}{2}$.	$\frac{1}{2}$.	$\frac{3}{4}$	final.
A	17.3	21.00	19.0	20.20	26.10	25.70
B	19.05	25.90	26.0	24.70		
C	29.89	24.90	—	—	19.60	18.4

It is interesting to note that the values obtained by different methods are of the same order and do not differ much from one another considering the experimental difficulties. Now the values calculated from the p_H of the sol are expected to be in larger error. The values obtained from $\frac{1}{2}$, $\frac{3}{4}$ and final neutralisation points of NaOH titration curves are liable to be in error also because the slopes of the curves at these points are considerable.

The values from specific conductivity data and from the half neutralisation point are less liable to error and the mean of these values is 21×10^{-12} .

FIG. 1.

Sol A.



In order to evaluate therefore the dissociation constant of the acid, S_u , the concentration of the undissociated acid in equilibrium with the solid phase must be known. Stearic acid is a weak acid and further is present mostly in the solid insoluble state and under these conditions therefore S_u can be taken to be equal to the solubility of the acid without any serious error. The solubility of stearic acid as obtained by various workers are widely divergent and are reproduced below for comparison in Table II.

TABLE II.

Author.	Reference	Solubility.
Moore, Hutchinson and Wilson	<i>Biochem J</i> , 1909, 12 , 347.	$3.5 \times 10^{-3}N$ at 37° .
Siedell	Bull. No 67 <i>Hygienic Laboratory U S Public Health Service</i> .	$1.2 \times 10^{-4}N$ at 25° .
McBain & Peaker	<i>Pro Roy Soc.</i> , 1929, A , 128, 394.	$4.1 \times 10^{-7}N$ at 25° .

It was therefore thought desirable to determine the solubility of the acid. Large quantities of stearic acid hydrosol and also conductivity water saturated with stearic acid by shaking or by keeping at $60-65^\circ$ for several hours and then cooling to the desired temperature, were ultrafiltered through 'Cella' finest membrane. The filtrate was evaporated and weighed from a platinum bowl in small instalments. The results obtained are given below in Table III.

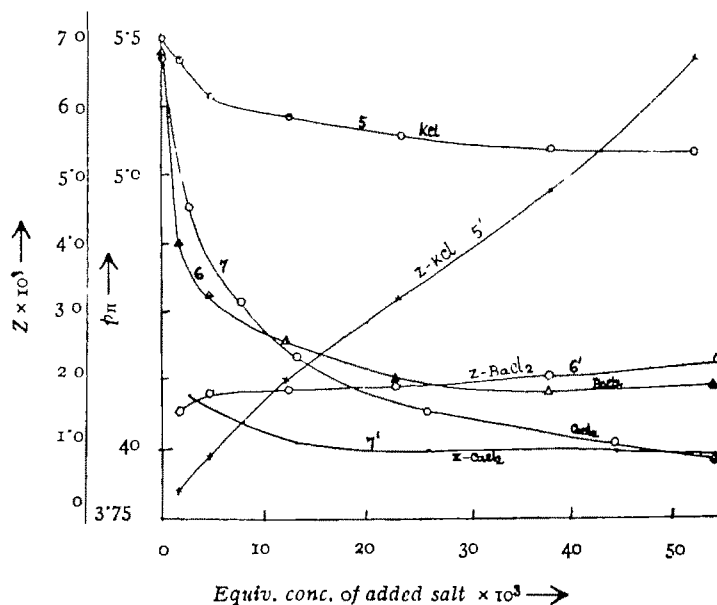
TABLE III

No of expts	Solubility of the acid.	No of expts	Solubility of the acid.
1	$1.05 \times 10^{-5}N$	3	$1.19 \times 10^{-5}N$
2	1.16	4	1.18

On dividing the mean value of $K S_u$ by the mean value of the solubility, the dissociation constant of stearic acid becomes 1.77×10^{-6} at 35° . The value obtained as a second approximation from $[H^+]$ calculated from the solubility and dissociation constant thus obtained is 1.3×10^{-6} .

FIG. 2.

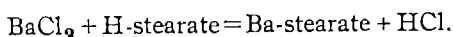
Sol C.



Interactions with Neutral and Hydrolysable Salts.

(a) *Interactions with Neutral Salts — p_H change.*—On the addition of KCl, BaCl₂ and CaCl₂, the p_H diminishes (Fig. 1, curves 1, 2, 3, 4; Fig. 2, curves 5, 6, 7; Fig. 4 curves 14 and 15). Compared to the bivalent cations calcium and barium, potassium has a relatively smaller effect. BaCl₂ produces a steeper initial drop in p_H but at higher concentration CaCl₂ appears to be relatively more effective. This observation is similar to that reported by Mukherjee, Mitra and Mukherjee (*Trans. Nat. Inst. Sci. India*, 1937, 1, No. 10, p. 227), in the case of silicic acid and hydrogen clay sols where the difference has been attributed to the greater coagulating effect of BaCl₂.

The liberation of hydrogen ions on the addition of neutral salts is the result of the formation of either (i) the corresponding stearate in the solid state or (ii) undissociated salt molecules, adhering possibly by lattice forces to the surface of the particles of the acid. The barium or calcium salts being less soluble than the potassium salts, BaCl₂ or CaCl₂ have greater power for lowering the p_H . The analysis of the ultrafiltrate shows that the reaction that takes place is, however, far from being complete, only a relatively small proportion of stearic acid reacts with the salt and that HCl is produced by the reaction :



If solid stearates are formed the following reactions are expected at constant temperature and pressure:

$[\text{H}^+][\text{Stearate}^-] = S$, $[\text{K}^+][\text{Stearate}^-] = S_1$, $[\text{Ba}^{++}][\text{Stearate}^-]^2 = S_2$ and $[\text{Ca}^{++}][\text{Stearate}^-]^2 = S_3$ where S , S_1 , S_2 and S_3 represent the ionic products of stearic acid, potassium stearate, barium stearate and calcium stearate respectively.

Therefore, $[\text{K}^+]/[\text{H}^+] = S_1/S = Z_1$,

$$\sqrt{[\text{Ba}^{++}]} / [\text{H}^+] = \sqrt{S_2}/S = Z_2,$$

and

$$\sqrt{[\text{Ca}^{++}]} / [\text{H}^+] = \sqrt{S_3}/S = Z_3.$$

The calculated hydrogen ion concentrations are liable to a greater error than the p_H values. The concentration of barium ions have been obtained by subtracting half of the increase of the concentration of hydrogen ions from the molar concentration of barium chloride added to the sol.

Values of these ratios calculated from curves 1 and 3 are plotted in curves 1' and 3' in Fig. 1 and from curves 5, 6 and 7 in curves 5', 6' and 7'

in Fig. 2. For BaCl_2 and CaCl_2 , the 'Z' values after an initial variation tend to become constant, showing that solid calcium and barium stearates are formed. For KCl, however, the ratio varies continuously with the concentration of the salt. The latter probably forms an acid stearate or normal stearate which does not form a separate phase but is stable at a certain p_H .

It also appears from the 'Z' curves that complete equilibrium between the solid phases and the solution is difficult of attainment during the titration. From the 'Z' values the solubilities of barium and calcium stearates can be calculated. As already put forward

$$Z = \frac{\sqrt{S_2}}{S} = \sqrt{\frac{[\text{Ba}^{++}]}{[\text{H}^+]}}$$

and since S and Z are known the value of S_2 , the ionic product of barium stearate and thence the solubility of barium stearate can be calculated. The solubility thus obtained from different Z values are given in Table IV.

In a similar way the solubility of calcium stearate has been found out (Table IV, columns 4 and 5).

TABLE IV.

Sol.	Z values.	Solubility of Ba-stearate at 35°.	Z values.	Solubility of Ca-stearate at 35°.
A	2.63×10^3	$9.12 \times 10^{-6}N$	1.57×10^3	$6.45 \times 10^{-6}N$
	2.61	9.08	1.42	6.05
	2.45	8.7	1.51	6.3
C	2.5	8.83	1.5	6.28
	2.63	9.12	1.4	6.02
	2.74	9.3	1.34	5.83

(b) *Interactions with Hydrolysable Salts.*—A distinction between exchange and hydrolytic acidities of soil developed on treating an acid soil with neutral and hydrolysable salts is usually made. Experiments with pure systems are likely to be helpful for a proper understanding of the theoretical basis of this distinction. Variations of H-ion activities of stearic acid sols on progressive additions of hydrolysable salts have been studied with this object in view.

FIG. 3.

Sol B.

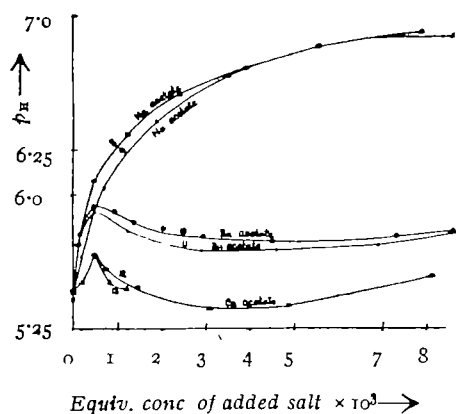
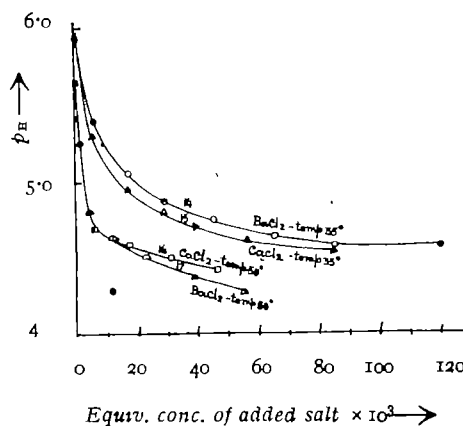


FIG. 4.

Sol E.



Stearic acid sol C was titrated with sodium acetate, barium acetate and calcium acetate solutions. The salt solutions were in each case made neutral by the addition of the requisite amount of acetic acid before use.

The titration curves are shown in Fig. 3 (curves 8 and 9 with Na salt, 10 and 11 with Ba salts and 12 and 13 with Ca salts). With sodium acetate p_H increases continually and gradually approaches the p_H of the added neutral acetate. With barium and calcium acetates on the other hand the p_H at first passes through a maximum, then diminishes and finally increases slowly. The maxima and the middle of the diffuse minima occur at $5 \times 10^{-4}N$ and $3.4 \cdot 5 \times 10^{-3}N$ respectively of both the added salts. The first value is somewhat less than the amount of the acid in the sol which is $6.5 \times 10^{-4}N$. The p_H at the maximum is 5.90-5.92 for Ba-acetate and 5.65 for calcium acetate. The curves are rather interesting. With increasing concentrations of barium and calcium ions insoluble stearates are formed and acetic acid is set free. This causes a lowering of the p_H , the rate of increase of free hydrogen ions falls off with the progress of the titration and the buffer action of the added salt, which has a higher p_H (7.0) becomes predominant. The p_H then rises slowly tending to approach the p_H of the acetate solution.

The Effect of Temperature on p_H , Dissociation Constant and Solubility of the Acid, Nature of the Titration Curves with Bases and Neutral Salts and the Solubility of Barium and Calcium Stearates.

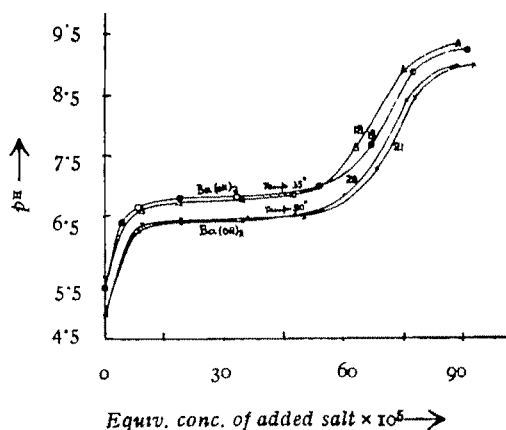
The solubility of stearic acid increases with temperature. The solubility has been determined at 50° and has been found to be $5.77 \times 10^{-5} N$. In stearic acid sols the colloidal particles constitute the solid phase and the intermicellary liquid contains its saturated solution. The hydrogen ion concentration calculated from the solubility of the acid at 35° agrees fairly well with the observed hydrogen ion concentration of the sol; the observed hydrogen ion concentration for different sols and that calculated from the solubility are given in Table V.

TABLE V.

Sol.	Obs.	Calc. from solubility.
A	$3.9 \times 10^{-4} N$	$3.7 \times 10^{-6} N$
B	4.2	—
C	4.3	—
D	3.0	—

FIG. 5.

Sol D



It appears therefore that the acid in solution is mainly responsible for the p_H of the solution. It is to be expected therefore that when with rise of temperature the solubility of the acid increases, the p_H of the sol should diminish. In order to verify this the p_H values of sols have been determined at 50° and it has been found that the p_H actually diminishes. The results obtained are given below in Table VI.

TABLE VI.

Sol R	p_H of the sol	
	at 35° .	at 50° .
Sol R	5.36	4.89
—	5.38	4.90
—	5.44	4.92

In stearic acid sols the whole of the acid reacts with the base and it is evident therefore that the total acidity of the sol should not change with rise of temperature. This is what has been experimentally found out (Fig. 5, curves 18, 19, 20, 21). The only difference that is observed is that the middle flat portion of the curve occurs at a lower p_H at higher temperatures.

The dissociation constant of the acid at 50° calculated from the hydrogen ion concentration of the sol at the temperature by the method already described gives the value, 2.8×10^{-6} .

TABLE VII.

Sol E.			
Z values of $BaCl_2$ titrations	Solubility of Ba -stearate at 50° .	Z values of $CaCl_2$ titrations.	Solubility of Ca -stearate at 50° .
4.7×10^3	2.19×10^{-5}	5.08×3	2.29×10^{-5}
4.94	2.26	5.5	2.4
4.64	2.18	5.00	2.28

Stearic acid sol E has been titrated at 50° with neutral barium and calcium chlorides (Fig. 4, curves 16 and 17) and the 'Z' values calculated from these curves are given in Table VII. The solubility of calcium and barium stearates calculated from these 'Z' values are also given in Table VII.

In conclusion, I take this opportunity to offer my sincere thanks to Prof. J. N. Mukherjee, D.Sc., for his kind interest in the work. Thanks are also due to the University of Calcutta for awarding me a research scholarship during the tenure of which this work was done.

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Received September 20, 1939.

STUDIES ON THE ESSENTIAL OIL FROM THE RHIZOME OF *ACORUS CALAMUS*. PART I. ISOLATION AND EXAMINATION OF CALAMOL.

BY MUHAMMAD QUDRAT-I-KHUDA, ASUTOSH MUKHERJEE AND
SUBASH KUMAR GHOSH.

From the rhizome of *Acorus calamus*, an oily substance, calamol ($C_{15}H_{18}O_3$) has been extracted. It is found to be an allyltrimethoxybenzene derivative and it yields a tetrabromo derivative. Calamol by the action of alkali isomerises into isocalamol, both the isomers yielding on oxidation the same calamonic acid ($C_{10}H_{12}O_6$), a trimethoxybenzoic acid, isomeric with asaronic, trimethoxypyrogallic or trimethylgallic acid, but not identical with any of them.

The European variety of acorus has been examined by various workers with different results. In the present investigation the rhizomes of the variety locally known as "ghore bacha" has been used.

Kurbatow (*Ber.*, 1873, **6**, 1210) distilled an oil from *Acorus calamus* and considered it to be $C_{10}H_{18}$. Von Soden and Rajahn (*Pharm. Z.*, 1901, **46**, 243) isolated a solid, calameon, ($C_{15}H_{26}O_2$) from the Galician root. Thoms and Beckstroem (*Ber.*, 1901, **34**, 1021; 1902, **35**, 3187, 3195; *Ber. Phar. Ges.*, 1902, **12**, 257) isolated asarone (m.p. 61°), asarylic aldehyde, eugenol, *n*-heptylic ester and some terpenes from the Japanese oil. Semmler and Spornitz (*Ber.*, 1913, **46**, 3700) examined the Russian oil from which they obtained pinene, camphene, camphor and also a sesquiterpene ($C_{15}H_{24}$). Schimmel & Co. determined the physical constants of the Japanese oil (Schimmel's Reports, April, 1909, p. 22). Russel (*J. Amer. Chem. Soc.*, 1915, **37**, 2387) examined the oil obtained from the plants cultivated in Madison, Wisconsin.

Some work has also been done on the Indian Calamus oil. Rao, Sudborough and Watson (*J. Indian Inst. Sci.*, 1925, **8A**, 149) obtained 1.5 to 3.5% of oil from the roots, procured from Coimbatore district, and determined its constants. In recent years Kelkar and Rao (*ibid.*, 1934, **17A**, 25) described an oil obtained from *Acorus calamus* that contained such compounds as palmitic and heptonic acid, ester of palmitic acid together with some pinene, camphene, asaraldehyde, eugenol, asarone, calamene, calamenol and calameon. But the rhizome that we have used appears

to behave differently. It gives an oil that does not appear in any way similar to any of the substances mentioned above ; on the other hand it appears to be similar to some extent to the compound isolated by Rao and Subramanian (*Current Science*, 1935, 8, 552), but is not identical with it.

The oil isolated in a yield of about 8% of the dried root gives only one product which boils at almost a constant temperature and we could not isolate the fractions mentioned by Rao, Sudborough and Watson (*loc. cit.*) or by Kelkar and Rao (*loc. cit.*). To this compound, we have given the name "calamol".

Calamol is a colourless mobile liquid, with a strong characteristic and a rather pleasant aromatic odour. It does not show the presence of any acids, esters, etc. The M. W. and analytical values of the sample suggest its formula to be $C_{13}H_{16}O_3$. It is most probably a benzenoid compound, isomeric with asarone.

The oil is transformed into an isomeric substance when boiled with alcoholic potassium hydroxide and no trace of any acid could be isolated from the alkaline solution. This neutral oil has odour reminiscent of the aroma of "Bel fruits" (*Aegle marmelos*). We conclude that calamol is not an ester and it does not possess a carboxyl group, but is isomerised with alkali into isocalamol.

The ready absorption of bromine by the compound indicates the presence of an unsaturated group in the molecule. The bromo derivative could not be obtained pure, as it decomposes on distillation. An analysis of the crude bromo compound suggests it to be a tetrabromo derivative. In presence of palladium, calamol takes up two atoms of hydrogen and gives dihydrocalamol ($C_{12}H_{18}O_3$).

The original oil, calamol, can thus be written as



which changes into isocalamol $C_9H_{11}O_3 - CH = CH \cdot CH_3$.

Aluminium chloride decomposes calamol partially with the production of a phenolic compound of a characteristic phenolic odour. Methoxyl determination indicates three methoxyl groups in calamol, hence it is probably a trimethoxyallylbenzene.

isoCalamol also analyses for three methoxyl groups, therefore, the action of alkali is restricted to the allyl group of the molecule only.

Calamol on oxidation should give a trimethoxyphenylacetic acid, but when it is oxidised by potassium permanganate solution calamonic acid

(trimethoxybenzoic acid) is formed, the double bond being shifted by the alkali of the reaction mixture. The m.p. of the product precludes its being trimethoxypyrogallol carboxylic acid, trimethoxygallic acid, trimethylphloroglucinol carboxylic acid and 2,4:5-trimethoxybenzoic acid (asaronic acid).

It can be 2:4:6-trimethoxy- or 3:4:6-trimethoxybenzoic acid. On demethylation of the acid, a trihydroxybenzoic acid is obtained which melts at a temperature different from the m.p. of phloroglucinol carboxylic acid or 2:4:5-trihydroxybenzoic acid.

Therefore, the relative orientation of the methoxy groups is probably 2:3:5 or 2:3:6 or 3:4:6. Experiments are now in progress to decide the question.

EXPERIMENTAL.

Extraction of Calamol from Ghore Bacha.—The rhizome as obtained from the market, without any dressing, was cut into very small pieces and crushed as fine as was practicable. The crushed rhizome (200 g.) was then mixed with 400 c.c. of water, and was distilled in steam for 6 hours (direct heating on a sand-bath should be avoided to prevent charring of the fibres) and the total amount of distillate collected was 7 litres. This was saturated with common salt and was extracted twice with ether. The ethereal extract was dried (fused calcium chloride) and the solvent removed. The residual oil was fractionally distilled under reduced pressure. Calamol had b.p. 153-54°/5 mm.

The following quantities of the oil were obtained from different experiments:

(i) From 1000 g., 80 g (ii) from 1400 g., 108 g. (iii) from 1600 g., 125 g. (iv) from 1000 g., 81 g. of calamol were obtained, i.e. from a total quantity of 5000 g. of rhizome 394 g. of the essential oil were obtained constituting 7.88%.

The oil obtained as above was purified by several redistillations and the purest sample, b.p. 153-154°/5 mm., was analysed. [Found: C, 69.3; H, 8.3; M.W. (ebullioscopic method), 214. $C_{12}H_{10}O_3$ requires C, 69.2; H, 7.7 per cent. M.W., 208]. [Found: OMe, 44.7. $C_9H_7(OMe)_3$ requires OMe, 44.7 per cent]. It had $d_4^{20.1}$, 1.07021; $n_D^{20.1}$, 1.55012; Found: $[R_L]_D$ 61.9 (calc. 58.5).

Conversion of Calamol into isoCalamol.—To a solution of potassium hydroxide (20 g.) in water (30 c.c.) was added calamol (20 g.) and enough rectified spirit (100 c.c.) to make a clear and homogeneous solution, which was then heated under reflux for about 10 hours and the alcohol was distilled off. The cooled mixture was diluted with water and extracted with ether. The alkaline solution furnished nothing definite when acidified and extracted in the usual way. The ethereal extract was washed with water and then dried and distilled, b.p. $133^{\circ}/2$ mm. It possessed a very characteristic odour which was absolutely different from that of the original oil. [Found : C, 69.3; H, 7.8; OMe, 44.9; M. W. (cryoscopic), 208.1. $C_{12}H_{18}O_3$ requires C, 69.2; H, 7.7; OMe (3 methoxyls), 44.9 per cent]. It had $d_4^{31.5}$, 1.07261; $n_D^{31.5}$, 1.55229 whence $[R_L]_D$, 61.85 (calc. 58.85).

Action of Bromine on Calamol.—Bromine was rapidly absorbed when a dry petroleum solution of bromine (1.5 g.) was added to a solution of calamol (3 g.) in the same solvent (100 c.c.). A heavy deeply coloured bromo compound separated out, the supernatant petroleum solution was removed and the coloured mass was taken up in ether and washed with a dilute solution of potassium hydroxide, charcoaled and filtered. The nearly colourless solution was freed from the solvent and the residual mass was left in *vacuo*, dark blue colour developing on standing. On distillation it rapidly decomposed and no definite compound could be isolated. The crude bromo compound was analysed after keeping in the desiccator for some time. [Found : Br (Piria and Schiff), 57.1. $C_{12}H_{11}O_3Br_4$ requires Br, 60.8 per cent].

Catalytic Hydrogenation of Calamol.—Calamol (22 g.) in absolute alcohol (100 c.c.) was reduced with 1% palladium chloride (50 c.c.) containing gum arabic (10 c.c. of 5%) when the absorption was quite rapid in the beginning, 2.5 litres of hydrogen being absorbed in about $1\frac{1}{2}$ hours. The reaction was carried on for $5\frac{1}{2}$ hours. The mixture was diluted with water till turbid and then extracted with ether, the ethereal extract washed with water and dried (calcium chloride) and solvent removed. Dihydrocalamol boiled at $124^{\circ}/2$ mm. (yield 20 g.). (Found : C, 68.72; H, 8.72. $C_{12}H_{18}O_3$ requires C, 68.6; H, 8.6 per cent). It had $d_4^{31.2}$, 1.03109; $n_D^{31.2}$, 1.51219 whence $[R_L]_D$, 61.1 (calc. 58.0).

Demethylation of Calamol (a) Demethylation with Aluminium Chloride.—Calamol (20 g.), dissolved in dry petroleum ether (100 c.c., b.p. $50-70^{\circ}$) was demethylated with anhydrous aluminium chloride (20 g.) at 100° for 5

hours. The cooled mass was extracted with petrol, solvent was removed and the residue was distilled under reduced pressure. The phenolic compound had b.p. $115^{\circ}/2$ mm. (Found : C, 68.1, H, 8.37. $C_{11}H_{14}O_3$ requires C, 68.04 ; H, 7.2 per cent). Hence it seems that aluminium chloride causes partial demethylation, only one methoxyl group being lost.

(b) *Demethylation with Hydriodic Acid*.—For complete demethylation calamol (10 g.) was heated with hydriodic acid ($d\ 1.7$) for $2\frac{1}{2}$ hours at $115-120^{\circ}$. The cooled mixture was thoroughly extracted with ether, the ethereal extract washed first with water, then with a little dilute solution of sodium thiosulphate and finally with water. After drying over sodium sulphate and the removal of solvent a black tarry residue remained which could not be purified by charcoaling in ethereal solution. It was extracted with water and then kept in contact with animal charcoal for some time. After the removal of water under reduced pressure, only a small quantity of an oily residue was obtained. The oil decomposed on distillation under reduced pressure (2 mm.). It was, therefore, benzoylated in the usual way and a solid substance was obtained which was purified by crystallisation from dilute rectified spirit. After several crystallisations, it had m.p. 96° . (Found : C, 75.0 ; H, 4.1. $C_{30}H_{22}O_8$ requires C, 75.3 ; H, 4.6 per cent).

Oxidation of Calamol : Preparation of Calamonic Acid.—A mixture of calamol (25 g.) sodium hydroxide solution (400 c.c. of 10%) was oxidised with the gradual addition of a solution of potassium permanganate (140 g.) in water (3500 c.c.) with stirring for 6 hours and then the mixture was allowed to stand overnight. The clear filtrate was concentrated on a water-bath till crystallisation set in. A thick flocculent precipitate, obtained by acidification with hydrochloric acid, was crystallised from hot water in fine colourless needles, m. p. 143° . [Found : C, 56.3 ; H, 5.6 ; OMe, 41.36. M.W. (by titration), 211.8. $C_{10}H_{12}O_6$ or $C_6H_2(OMe)_3COOH$ requires C, 56.6 ; H, 5.6 ; OMe, 41.3 per cent. M.W. 212].

Calamol was also oxidised under condition exactly similar to that described above and gave the same compound, with identical m.p. and mixed m.p.

Demethylation of Calamonic Acid : Preparation of Trihydroxybenzoic Acid.—The foregoing trimethoxybenzoic acid (10 g.) was heated with hydriodic acid ($d\ 1.7$) under conditions similar to those described above in the case of the allyl compound when crystalline solid, mixed with some oily and coloured impurities, was obtained. After draining on a porous plate, the

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acid was crystallised from a mixture of dry ether and petrol in the colourless form, m.p. 97° . (Found . C, 49.2 , H, 3.6 $C_7H_6O_3$ requires C, 49.4 ; H, 3.6 per cent).

Our thanks are due to Mr. Ghanimat Ali of [•]Islamia College, Calcutta who assisted us in the extraction of some calamol.

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Received August 18, 1939,

THE ELECTROCHEMICAL PROPERTIES OF COLLOIDAL SILICIC ACID PART I INTERACTION WITH BASES *

By. B. CHATTERJEE.

The interaction of silicic acid sols with dilute and concentrated bases has been studied. A comparison of the free and total acidities (at the first inflexion point) of the sols and those of their ultrafiltrates shows that the sols behave as strong acids. Titration curves with different dilute bases [NaOH, Ba(OH)₂, Ca(OH)₂] show inflexion points (first inflexion point) indicating full neutralisation of an acid between p_H 4.5 and 5.6 [with NaOH between p_H 5.17 and 5.4, with Ba(OH)₂ between p_H 4.87 and 5.6 and with Ca(OH)₂ between p_H 4.5 and 5.5.] A comparison of the slopes of the titration curves shows that the intensities with which different bases react with the sol are in the order Ca(OH)₂ > Ba(OH)₂ > NaOH but the amount of acid neutralised by these bases at each of the inflexion points is the same. The potentiometric titration curves of the sol with concentrated solutions of NaOH show second inflexion points between p_H 11.0 and 11.70. The lower the silica content of the sol, the lower is the p_H at which the second inflexion occurs. The total acidity, expressed in normality per litre, calculated from the second inflexion point, shows fair agreement with the silica content of the sol expressed in g mols per litre. The evidence shows that the salt NaHSiO₃ is formed at the second inflexion point. The ultrafiltrates of different sols contain different amounts of dissolved silicic acid. The buffer capacity curves show only one maximum near about the point of half neutralisation. This maximum value is considerably greater than that of an acid in true solution.

This paper deals with some aspects of the interactions of silicic acid sols with bases which have not been covered by previous investigators on this subject. The potentiometric titration curves of a number of carefully purified silicic acid sols have been obtained and the features of the curves, that is, their slopes and buffer capacities in different p_H regions, examined.

The sols were prepared by the action of chemically pure hydrochloric acid on solutions of sodium silicate (Merck's pure quality and dialysed in parchment bags against repeated changes of distilled water till the dialysate gave no test for chloride with silver nitrate). The sols L' and P were then electrodialysed for effecting further purification. The membranes were purified as described by Rabinowitsch and Kargin (*Trans. Faraday Soc.*, 1935, **31**, 289). The general experimental arrangements, the method of titration and the apparatus used were the same as those described in a previous paper from this laboratory (Mukherjee *et al.*, *Indian J. Agric. Sci.*,

* The results given in this paper have been taken from the annual reports (1936-37, 1937-38, 1938-39) submitted to the Imperial Council of Agricultural Research, India.

1936, 6, 517). It was considered desirable to check the initial p_H values obtained with the hydrogen electrode with those obtained with a different electrode, *e.g.*, the glass electrode. A Morton type glass electrode in conjunction with a Cambridge valve potentiometer was used.

The Reproducibility of the E. M. F. of the Hydrogen Electrode.

It has been previously observed in this laboratory (Mukherjee *et al.*, *loc. cit.*) that the initial p_H values of the sol, as measured from the E.M.F. of the hydrogen electrode show irregular variations. These variations, however, did not materially affect the nature of the titration curves or the total acidity values calculated from them. On cleansing and replatinising the electrodes more concordant p_H values were often obtained with this sol. The extent to which reproducible values of the p_H can be obtained has been examined. The electrodes were cleansed and replatinised each time before use. The results are given in Table I.

TABLE I.

Sol.		p_H .						Average p_H .
K	Hydrogen electrode	4'14	4'05	4'02	3'92	3'90	4'00	4'00
	Glass electrode	3'96	3'97	4'00	4'02			3'99
L	Hydrogen electrode	4'47	4'45	4'44	4'46	4'44	4'43	4'45
			4'45	4'43				
	Glass electrode	4'45	4'40	4'40	4'50			4'44
L'	Hydrogen electrode	3'97	4'04	3'98	3'99			4'00
P	Hydrogen electrode	4'73	4'70	4'67	4'70	4'67		4'69

The good agreement between the initial p_H values of the sols measured with the hydrogen electrode on different days and between the average from the hydrogen electrode with that from the glass electrode indicate, that the hydrogen electrode is serviceable at such low unbuffered hydrogen ion concentration.

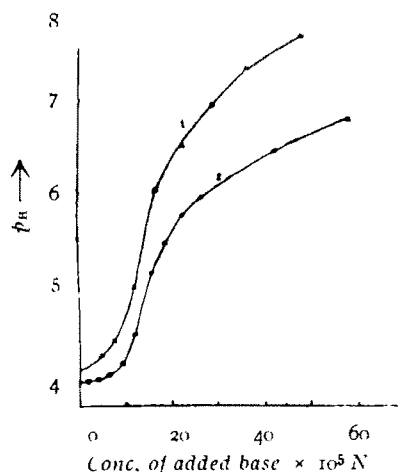
Interaction of Silicic Acid Sols with Dilute Bases.

The free and total acidities of silicic acid sols K, L', M, P and Q as well as of their ultrafiltrates are given in Table II (see also Figures 1, 2, 3). The

silica contents of the sols and the p_H at the inflexion points in the titration curves are also shown in the table.

FIG. 1.

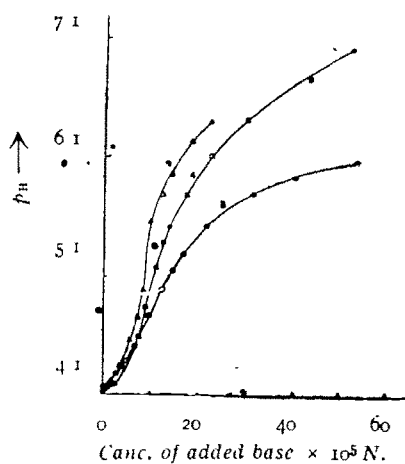
Sol L'.



Curves 1-2 refer respectively to Sol-
NaOH and Sol- $\text{Ca}(\text{OH})_2$

FIG. 2.

Sol M



Curves 3-5 refer respectively to
Sol-NaOH, Sol- $\text{Ba}(\text{OH})_2$ and Sol-
 $\text{Ca}(\text{OH})_2$

TABLE II.

Sol.	SiO_2 per litre.	Free acidity (normality).	p_H at first inflexion	Total acidity (normality)	Base used
Sol K	0.33 g. mol	10.00×10^{-5}	4.50	10.00×10^{-5}	$\text{Ca}(\text{OH})_2$
Ultrafiltrate of sol K	—	0.81	—	1.1	„
Sol L'	0.10	10.0	4.80	14.5	$\text{Ca}(\text{OH})_2$
Ultrafiltrate of sol L'	—	1.23	5.40	14.5	NaOH
			—	1.2	$\text{Ca}(\text{OH})_2$
Sol M	0.16	7.08	4.70	8.9	$\text{Ca}(\text{OH})_2$
			4.87	9.0	$\text{Ba}(\text{OH})_2$
			5.17	9.0	NaOH
Ultrafiltrate of sol M	—	0.68	—	0.75	$\text{Ba}(\text{OH})_2$
Sol P	0.026	2.0	5.5	3.8	$\text{Ca}(\text{OH})_2$
Ultrafiltrate of sol P	—	0.2	5.6	3.8	$\text{Ba}(\text{OH})_2$
			—	—	Nil
Sol Q	0.26	7.9	4.84	14.5	$\text{Ca}(\text{OH})_2$
Ultrafiltrate of sol Q	—	0.89	4.96	14.5	$\text{Ba}(\text{OH})_2$
			—	1.2	„

A comparison of the total acidities of the sols and their ultrafiltrates shows beyond doubt that colloidal silicic acid possesses an intrinsic acid character independent of the presence of dissolved acids in the system. Sol P shows a very low total acid, compared to other sols. It is to be noted that the silica content of the sol is much lower than that of the other sols. This probably explains the very weakly acidic properties observed by Rabinowitsch and Kargin (*loc. cit.*) with sols having very low (0.02%) silica contents.

In agreement with previous observations from this laboratory (Mukherjee *et al.*, *Trans. Nat. Inst. Sci. India*, 1937, **1**, 227), the titration curves show inflexion points in the acid region. An inspection of the curves of Rabinowitsch and Laskin (*Z. physikal. Chem.*, 1928, **134**, 387) shows similar inflexion points in the acid region, between p_H 5.2 and 5.4. The occurrence of the inflexion point in the acid region indicates that the acid titrated may contain several dissolved acids having different dissociation constants and/or a polybasic acid. The negligible total acidity of the ultrafiltrate shows that the sol does not contain dissolved acid, other than silicic acid. If the sol behaved as a polybasic acid the titration curves may be expected to show further inflexion points at concentrations of the added base which are multiples of the total acid at the first inflexion point. The

FIG. 3.

Sol Q

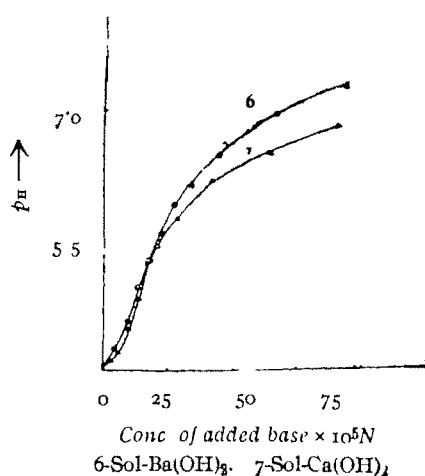
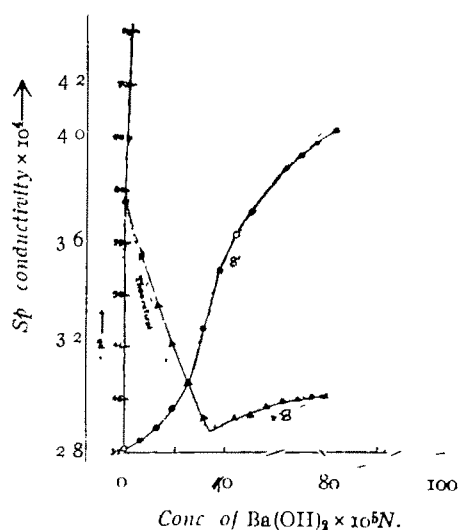


FIG. 4.

Sol E.



titration curves (*vide* curves 10, 12, 14, 16, 17) of silicic acid sols with concentrated alkali do not show a second inflexion point till a high p_H value, which lies between 11.7 and 11.0 depending on the concentration of silica in the sol, is reached; and it appears that the polybasic acid character, if any, is not evident within the range of p_H values used. The smooth nature of the curves (*vide* curves 11, 13 and 15) obtained by plotting buffer capacities against concentration of added alkali, supports this conclusion. The interaction of silicic acid sol with concentrated alkalis has been dealt with in a subsequent section (*vide* p. 589).

The potentiometric titration curves (*vide* curves 1, 2, 3, 4, 5, 6, 7, 8', 9') of the sols with $\text{Ba}(\text{OH})_2$, $\text{Ca}(\text{OH})_2$ and NaOH show inflexion points when equal amounts of the bases have been added. The inflexion points in the titration curves with different bases, however, do not lie at the same p_H . The inflexion point in the titration curve with caustic soda lies at the highest p_H and that in the calcium hydroxide curve at the lowest. An examination of the slopes of the curves shows that the calcium hydroxide curve has a flatter run than the baryta curves. The slope of the latter curve is again smaller than that of the NaOH curve. Thus the buffering effect and hence the intensity of the reaction is in the order: $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$. The greater effect of calcium hydroxide compared to $\text{Ba}(\text{OH})_2$ is in agreement with the greater insolubility of calcium silicate compared

FIG. 5.

Sol R

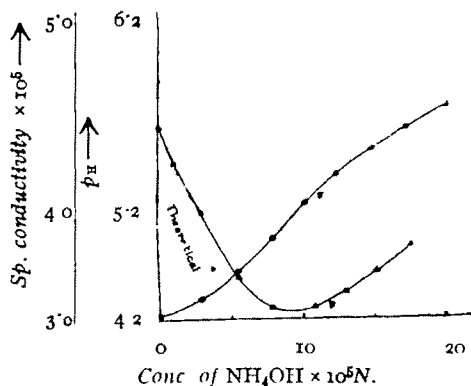
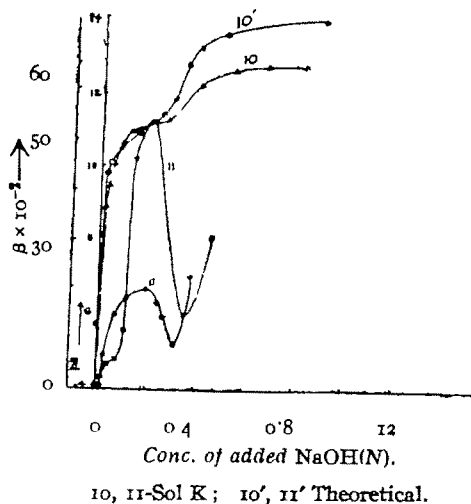


FIG. 6.



to barium silicate noted by Joseph and Hancock (*J. Chem. Soc.*, 1925, 127, 2813). Alternatively the salt molecule formed on the surface under these conditions by interaction with calcium hydroxide are more insoluble or stable than those formed with baryta. In the next paper of this series it will be shown that in the interaction of silicic acid sols with barium and calcium chlorides, on the other hand, the barium salt has a greater effect in that it liberates a larger amount of acid. It thus appears that the resulting salt molecules have different properties in the acid and alkaline regions, indicating two different types of reactions.

Previous results (Mukherjee *et. al.*, *loc. cit.*, 1937) show that the total acidity of a silicic acid sol, calculated from the first inflexion points in the titration curves with dilute bases, shows a fair agreement with that calculated from minima in the conductometric titration curves. This coincidence is characteristic of a strong acid. Moreover, it will be seen from the following table that the observed slope, $d\mu/dc$ (the rate of change of specific conductivity μ with concentration c of added bases) of the conductometric titration curves (*vide* curves 8, 9) do not differ widely in the initial part of the titration from the value calculated for the neutralisation of hydrochloric acid.

TABLE III.

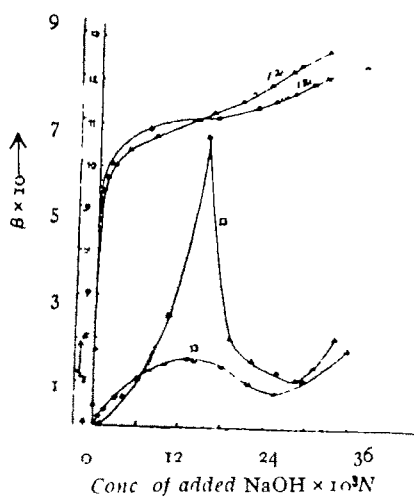
Sol.	Base used.	$d\mu/dc$	
		Calculated.	Observed
E	Ba(OH) ₂	0.326	0.330
F	"	"	0.263
R	NH ₄ OH	0.315	0.320

This observation is important in the light of a recent paper by Bruyn and Oberbeek (*Kolloid Z.*, 1938, 84, 816) who found that silver iodide sols contain foreign ions such as Cu⁺⁺ and Zn⁺⁺ coming from distilled water used for the purification by electrodialysis and electrodecantation. As a result they found that the potentiometric and conductometric titration curves indicate a perceptible buffer action which disappeared on using purer double distilled water. It will appear from the data cited above that no such disturbing foreign ions, Cu⁺⁺, Zn⁺⁺ or Al⁺⁺⁺ are present in the silicic acid sols used by us. The conductometric titration curves (*vide* curves 8, 9) show no such buffering in the initial stages of the titrations. Tests for Cu⁺⁺⁺ in the silicic acid sols by means of rubeanic acid in slightly ammoniacal medium also gave negative results.

*Interaction of Silicic Acid Sol with Alkali in
highly Alkaline Regions*

Reference has already been made (*vide* p. 586) to the occurrence of inflexion points in the acid region with dilute bases. Sols K, L, L', P and Q have been titrated with concentrated solutions of caustic soda upto nearly p_H 12.0. Titration with baryta and calcium hydroxide is not possible on account of coagulation.

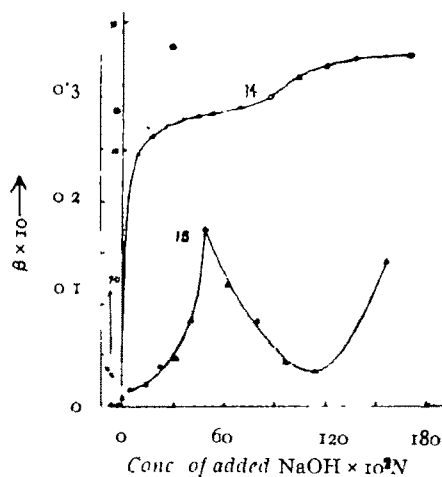
FIG. 7.



12, 13-Sol L : 12', 13'-Theoretical.

FIG. 8.

Sol L'.



The titration curves (10, 12, 14, 16, 17, 18) all show an inflexion point in the p_H region 11.0 to 11.70. The silica contents of the sols have been given in Table IV together with the p_H at the second inflexion.

TABLE IV

*Relation between silica content of the sol and the p_H of the
second inflexion.*

Sol	SiO ₂ per litre	p_H at the second inflexion point
K	0.33 g mol	11.65
L	0.27	11.70
Q	0.26	11.70
L'	0.10	11.45
P	0.027	11.05

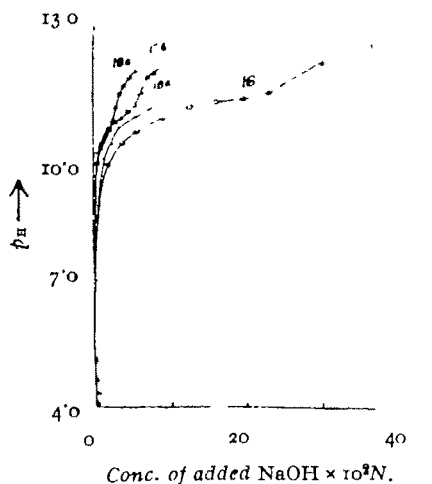
Table IV shows that the p_H at the second inflexion diminishes with the silica content of the sol. Treadwell and Wieland (*Helv. Chim. Acta*, 1930, **13**, 842) have also observed that the titration curves of sodium silicate solutions with hydrochloric acid show definite inflexion points near about p_H 11.0 and that this p_H varies with the concentration of the solution. The variation of the p_H at the second inflexion point with the silica content of the sol has been studied by titrating different dilutions of sol Q. The results are given below.

TABLE V.

Dilution	SiO ₂ per litre	p_H of the second inflexion	Total acidity at inflexion (in normality)
Pure sol	0.26 g. mol	11.70	0.26
1:2.5	0.10	11.55	0.10
1:4	0.065	11.35	0.06
1:10	0.026	11.00	0.027

It should, however, be mentioned that for small changes in the concentrations of the sols the change in the p_H of the second inflexion point is not appreciable (*vide* Table IV)

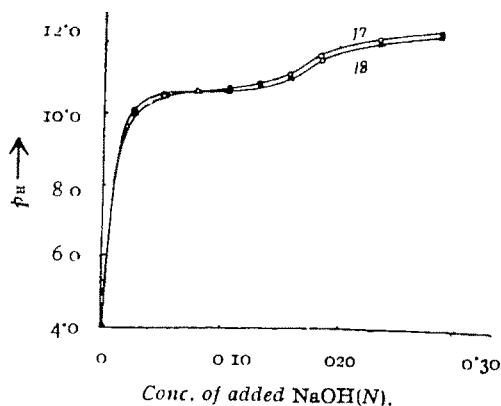
FIG. 9



16-Sol Q; 16a-Q/2.5; 16b-Q/4; 16c-Q/10,

FIG. 10.

Sol T



17-titration as usual
18 " in bottle,

Britton (*J. Chem. Soc.*, 1927, 425) observed inflexion points between p_H 10.0 and 11.0 when silicic acid, freshly prepared by the interaction of sodium silicate solutions with hydrochloric acid, was titrated with baryta and calcium hydroxide. Treadwell and König (*Helv. Chim. Acta*, 1933, 16, 468) prepared silicic acid sol by electrodialysing a solution of sodium silicate and titrated with caustic soda solution to nearly p_H 13. Their titration curve shows an inflexion point at p_H 11.8 and in the alkaline region is almost similar to curves obtained in the present works. Differences are, however, to be observed in the acid region. The titration curve obtained by them shows a strong buffering between p_H 6.0 and 7.0. The curves (*vide* curves 10, 12, 14, 16, 17, 18) given in this paper do not show a buffer action in this region. A second point of difference is that the titration curve of Treadwell and König does not show the strong acid character of the sol in the initial stages of its titration with a dilute base which has been noted in this work, as also by Rabinowitsch and Laskin (*loc. cit.*). This arises probably from the fact that they used comparatively concentrated alkali (0.1N) from the beginning, whereas, 0.01N-alkali has been added in small drops in this work to get the first inflexion.

Relation between Silica Content of the Sol and the Total Acid Neutralised at the Second Inflexion.

In the following table the total acidities of the sols neutralised at the second inflexion have been compared with their silica contents, determined by analysis.

TABLE VI.

Sol.	Total acidity at inflexion point	SiO ₂ per litre.
K	0.35 N	0.33 g. mol.
L	0.26	0.27
Q	0.26	0.26
L'	0.10	0.10
P	0.026	0.027

It will be seen from Table IV that the total acidity values, calculated from inflexion points in the alkaline region, show fair agreement with the silica contents of the sols.

The titrations of silicic acid sols with concentrated alkalis already referred to were completed within the course of a single day. Fresh

additions of alkalis were made after the η_{sp}/c was found to remain constant for 15 minutes and a complete titration generally took about 11 hours. To ascertain whether equilibrium was fully attained at each stage during the titration, 20 c.c. of sols Q and T were taken in each of a number of Jena bottles with glass stoppers and increasing amounts of alkalis were added and the bottles frequently stirred. A long interval (48 hours for sol Q and 70 hours for sol T) was allowed for the interaction and the p_H values were determined with the hydrogen electrode. The results are shown in Fig. 10 and Table VII.

TABLE VII.

Sol.	SiO ₂ per litre	p_H at second inflexion	Total acidity at second inflexion.
Q (Titration as usual)	0.26 g. mol	11.70	0.26N
Q (Titration in bottles)	,,	11.55	0.265
T (Titration as usual)	0.17	11.47	0.17
T (Titration in bottles)	,,	11.33	0.17

The total acidity values calculated from the second inflexion points are in close agreement with the silica content of the sols. The results also show that the time allowed for in the continuous titration curves is practically sufficient for the attainment of equilibrium.

The Composition of the System at the Inflexion Point at High p_H .

Table VIII shows the ratio $\text{Na}_2\text{O} \cdot \text{SiO}_2$ of different sols at the inflexion points at highly alkaline region

TABLE VIII.

Sol.	$\text{Na}_2\text{O} \cdot \text{SiO}_2$ at inflexion point.
K	1:1.88
L	1:2.07
L'	1:2.0
P	1:2.07
Q	1:2.0

Thus, at the inflexion points in the titration curves the values of the ratio $\text{Na}_2\text{O}:\text{SiO}_2$ do not appreciably differ from 1:2. This suggests that the composition of the system at the inflexion point may be either NaHSiO_3 or NaSi_2O_5 . The presence of only one maximum near about the point of half neutralisation in the buffer capacity curve (*vide* curves 11, 13, 15) shows definitely that the second inflexion point indicates only the first stage of neutralisation of the colloidal acid and that the composition of the system at this point is NaHSiO_3 . Treadwell and Wieland (*loc. cit.*) and Harman (*J. Phys. Chem.*, 1927, **31**, 611) in their electrometric titrations of solutions of sodium silicate of different concentrations obtained two inflexion points corresponding to the first and second stages of neutralisation of a dibasic acid. They concluded that the first inflexion points in the titration curves of solutions of sodium silicate with hydrochloric acid at about p_H 11.0 correspond to the formation of NaHSiO_3 .

A comparison of the slopes of the titration curves (curves 10, 12, 10' and 12') of the sols with those of hypothetical acids in true solution having corresponding total acidities and dissociation constant, calculated from the p_H at half neutralisation, shows that the inflexion points in the titration curves of the sols lie at a lower p_H than those in the case of acids in true solution and that at higher concentrations of added alkali the titration curve of the sol has a flatter run than that of the hypothetical acid. The stronger buffer action of the sol is due to its colloidal nature arising from the fact that a continuous solution of the colloidal particles takes place in this region. The particles thus act as a reservoir from which fresh quantities of the acid pass into true solution. The buffer capacities have been discussed in a subsequent section.

Dissociation Constant of Colloidal Silicic Acid.

The titration curves of silicic acid sols with concentrated NaOH resemble those of a weak monobasic acid in true solution. If we now assume that the whole of the silica in the sol is present in a state of true solution and that the molar concentration of silica present in the sol represents its total acidity, the dissociation constant of colloidal silicic acid can be calculated from given points in the titration curves according to Henderson's equation,

$$H = K \frac{a - b - H + OH}{b + H - OH} \quad \dots (1)$$

where 'a' represents the initial concentration of acid given by the molar concentration of silica in the sol; 'b', the concentration of added base; H, the hydrogen ion concentration calculated from given p_H in the titration curve and OH, the concentration of Hydroxyl ions. The results obtained are given in the following table.

TABLE IX.

I	II	III	IV	V
K	0.33	11.0	9.28	9.0
L	0.27	11.12	9.28	9.0
Q	0.26	11.17	9.28	9.0
Q diluted 1 : 2.5	0.10	10.88	9.28	9.0
L	0.10	10.88	9.28	9.0
Q diluted 1 : 4	0.065	10.91	9.28	9.0
P	0.027	10.54	9.28	9.0
Q diluted 1 : 10	0.026	10.41	9.28	9.0

Column I gives the reference number of each sol, column II, their silica contents in g. mol. per litre, column III, the p_K values calculated at the points of half neutralisation, and column IV and V, the p_K values calculated at the points of half neutralisation of acids in true solution having dissociation constants respectively of 5.2×10^{-10} (*vide* p. 591) and 10^{-9} (Hägg, *Z. anorg. Chem.*, 1926, **155**, 21) and the corresponding total acidities of the sols. The p_K values for dissolved silicic acid corresponding to this stage of neutralisation are given in Table IXA for comparison.

TABLE IXA.

Author.	First dissociation constant of silicic acid.	p_K .
Hägg (<i>loc. cit.</i>)	10^{-9}	9.0
Treadwell and Wieland (<i>loc. cit.</i>)	$10^{-9.7}$	9.7
Joseph and Oakley (<i>J. Chem. Soc.</i> , 1925 127 , 2813)	10^{-10}	10.0
Harman (<i>loc. cit.</i>)	4.2×10^{-10}	9.3

It appears that the p_K values calculated from the points of half neutralisation of the sols are not constant but have a tendency to decrease with decrease in the concentration of the sols. The values obtained in

the case of acids in true solution, on the other hand, are constant within the range of concentrations studied. This difference probably arises out of the assumption that the whole of the silica is present in a state of true solution. Also the p_K values calculated at the points of half neutralisation according to equation (1) are not in agreement with those found for dissolved silicic acid.

Assuming solid silicic acid to be in equilibrium with dissolved silicic acid and its ions and applying the law of mass action, its first dissociation constant can be calculated as follows

$$[H^+].[HSiO_3^-] = K_1.[H_2SiO_3] = K_1S \quad \dots (2)$$

where S is the concentration of dissolved undissociated silicic acid molecules in equilibrium with the solid phase. Since

$$[H^+] + [B^+] = [OH^-] + [HSiO_3^-]$$

where B represents the concentration of Na we have

$$[HSiO_3^-] = [H^+] + [B^+] - [OH^-]$$

and

$$[H^+]\{[H^+] + [B^+] - [OH^-]\} = K_1S.$$

Thus K_1S can be calculated from the p_H at a given point in the titration curve and the amount of base added. The values of K_1S thus calculated at the second inflexion points of sols K, L, Q, T, L' and P are given in Table X.

TABLE X.

Sol.	Silica content per litre.	p_H at second inflexion.	K_1S
K	0.33 g. mol.	11.65	7.57×10^{13}
L	0.27	11.70	4.93
Q	0.26	11.70	4.93
T	0.17	11.47	5.49
L'	0.10	11.15	3.28
P	0.027	11.05	2.05

K_1S should have a constant value if the solution were saturated with respect to an unequivocal solid phase. But it is found to decrease with a decrease in the concentration of the sol (except in the case of sol T).

The concentrations of dissolved silicic acid in the ultrafiltrates* of the sols were next determined using the method of Lucas and King (*J. Amer. Chem. Soc.*, 1928, 50, 2385). The colour developed on adding ammonium molybdate and sulphuric acid to the ultrafiltrate was matched against a standard picric acid colour in a Hellige colorimeter. The results are given below.

TABLE XI.

Sol.	SiO ₂ per litre.	SiO ₂ in the ultrafiltrate per litre (at 30°) (S).	p_H of ultrafiltrate.	Calc. p_H of the ultra-filtrate.	p_H .
Q	0.26 g. mol.	8.3×10^{-4} g. mol.	5.05	† 6.04	‡ 6.19
T	0.17	9.8	4.80	6.00	6.15
L'	0.10	6.8	4.93	6.09	6.23
P	0.027	4.7	5.70	6.17	6.32

If silicic acid has a definite solubility§ the amount of silicic acid determined in the ultrafiltrates of the sols should have a constant value. The results, however, show that the ultrafiltrates of the sols contain different amounts of silicic acid and is not related in a simple way to concentration. The following factors require consideration.

* 'Finest' cella ultrafilters were used.

† Calculated on the assumption that the first dissociation constant of silicic acid is 10^{-9} , the effect of the second one being insignificant.

‡ Calculated on the assumption that the first dissociation constant of silicic acid is 5.2×10^{-10} (*vide* Table XII).

§ Widely different values for the solubility of silica are found in literature. The most systematic work on the solubility of silica carried out so far is that of Lenher and Merrill (*J. Amer. Chem. Soc.*, 1917, 39, 26, 30). They found that one litre of water dissolves 0.428 g. of SiO₂ at 90° and 0.16 g. at 25°. What they measured, however, was "the amount of silica which passed from a non-filtrable to a filtrable state." It was not definitely known whether this amount went into true solution or became colloidal hydrogel of silicic acid. The mean value of the solubility of colloidal silicic acid obtained in this work is 0.045 g. per litre, a value which is considerably less than that obtained by them.

(i) Varying amounts of sodium silicate may be present in the different sols.

(ii) Several types of silicic acids may be produced during the formation of colloidal acids from the molecularly dispersed acid, these probably have different solubilities.

(iii) Polymerisation of the molecules of the acid may be inhibited at different stages as the kinetics of such reactions are known to be susceptible to many influences. Ultramicrons of various sizes will thus be formed. These have possibly different solubilities. The difference in p_K obtained by previous workers by titration of alkali silicates with strong acids probably arises out of this effect. The crystalloidal solubility of the colloidal particles is practically negligible but the sols may contain different amounts of dissolved silicic acid in equilibrium with the fine micelles of different silicic acids.

(iv) The method used for the determination of dissolved silica may not be sufficiently reliable.

It appears from the investigations of Treadwell and Wieland (*loc.cit.*) and Harman (*loc. cit.*) that sodium silicate is completely converted into silicic acid between p_H 5.0 and p_H 6.0. The sols have p_H values below 5.0 and therefore it is very likely that the sols used in this investigation do not contain any sodium hydrogen silicate as such. The discrepancies between the observed and calculated p_H values (Table XI) indicate that fine micelles have passed through the ultrafilter and these have free hydrogen ions associated with them. More probably it is due to a trace of hydrochloric acid.

Values of K_1 obtained by dividing K_1S by the corresponding values of S are given in Table XII.

TABLE XII.

Sol.	Silica content per litre.	S (g. mol.).	K_1S .*	K_1 .	Mean K_1 .
Q	0.26 g. mol.	8.3×10^{-4}	4.93×10^{-13}	5.9×10^{-10}	
T	0.17	9.8	5.49	5.6	5.2×10^{10}
L'	0.10	6.8	3.28	4.8	
P	0.027	4.7	2.05	4.4	

The values of K_1 given in the above table may be taken to be practically constant if one considers the wide differences in the values of K_1 , the

* From Table X.

first dissociation constant of dissolved silicic acid obtained by previous workers (from 10^{-9} to 10^{-10} , *vide* Table IXA). K_1 , however, decreases from 5.9×10^{-10} to 4.4×10^{-10} as the concentration of silicic acid present in the sol diminishes from 0.26 to 0.027 g. mol per litre. The small variations in the values of K_1 may be attributed to errors of measurements. The mean value 5.2×10^{-10} probably gives the true value for the first dissociation constant of silicic acid.

The difference in p_K , given in Tables IX and that obtained by previous workers by titration of alkali silicates with strong acids arises out of a difference in the mode of reaction of silicic acid sol in the colloidal state. A higher energy appears to be necessary to dissolve the silicic acid molecules from the colloidal particles and the maximum buffering, characteristic of the slope near half neutralisation shifts to a higher p_H and consequently indicates a higher p_K . Calculation of buffer index will consequently be of value in understanding the nature of the interaction.

Buffer Capacity of Silicic Acid Sols.

The buffer capacities ($\Delta B/\Delta p_H$) at different points in the titration curves (*vide* curves 11, 13 and 15) of the sols K, L, and L' have been plotted against the equilibrium concentration of added alkali. They have been compared with those of a hypothetical acid, having the same dissociation constant and total acidity as the corresponding sol. The theoretical buffer capacities have been calculated according to the following equation (Slyke, *J. Biol. Chem.*, 1922, **22**, 525).

$$\beta = 2.303 \frac{a \cdot K \cdot H}{(K + H)^2},$$

where β represents the buffer capacity.

a is the total acidity determined by the stoichiometric concentration of SiO_2 present in the sol.

K is the dissociation constant determined from the p_H at the point of half neutralisation.

H represents the hydrogen ion concentration.

The buffer capacity curves (*vide* curves 11, 13, 15) are smooth and show only one maximum near about the point of half neutralisation. The

presence of only one maximum in the buffer capacity curves indicates that only the first stage of neutralisation has been reached up to the second inflexion point.

The maximum values of the buffer capacities of the sols near about the point of half neutralisation, have been compared with those of the corresponding hypothetical acids. The results are given in Table XIII.

TABLE XIII.

Sol.	SiO ₂ per litre.	Maximum buffer capacity of sol	Corresponding theoretical value in true solution
K	0.33 g mol.	0.54	0.20
L	0.27	0.67	0.15
Q	0.26	0.45	0.14
L'	0.10	0.172	0.058
P	0.027	0.035	0.015

Thus the values of the maximum buffer capacities in the titration curves of the sols are considerably greater than those in the theoretical curves. The greater buffer capacity observed in the case of the sols arises from a continuous solution of the colloidal particles, which act as a reservoir from which fresh quantities of acids are generated.

C O N C L U S I O N .

The hydrogen electrode when cleansed and plantinised before each experiment gives fairly reproducible p_H values of silicic acid sols. Silicic acid possesses an intrinsic acid character. Analysis of the ultra-filtrates shows that acids are not present as impurities in quantities to account for the form of the titration curves.

Titration curves of silicic acid sols with different bases show inflexion points at the same concentration of added bases. The buffering action indicated by the slopes of the titration curves, however, indicate that the relative intensities with which the different bases react with the sol are in the order: $\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$.

Titration curves of silicic acid sols with concentrated alkali show a second inflexion point at a high p_H value. The p_H at the second inflexion diminishes with the concentration of silicic acid in the sol. The total

acidity calculated from this inflexion point shows a fair agreement with the silica content of the sol. The p_K value of the sol calculated at the points of half neutralisation according to Henderson's equation is not in agreement with those found for dissolved silicic acid.

The ultrafiltrates of different sols contain different amounts of silicic acid. These variations have been attributed to the presence of (i) varying amounts of fine micelle and (ii) ultramicros of various sizes having different solubilities in the different sols.

The values of K_1 , obtained by dividing K_1S , calculated according to the equation

$$K_1S = [H^+] \{ [H^+] + [B^+] - [OH^-] \}$$

by S , the content of dissolved silicic acid estimated in the ultrafiltrate of the sol are of an order similar to that obtained by previous workers from titrations of silicates by acids.

The buffer capacity curve shows only one maximum near about the point of half neutralisation. The maximum value of the buffer capacity is considerably greater than that of the neutralisation curve of a dissolved acid having the same total acidity and dissociation constant as the sol.

The nature of the interaction of silicic acid sols with bases has been discussed.

The author takes this opportunity to offer his thanks to Prof. J. N. Mukherjee, D Sc., for his suggestions and advice, to the Imperial Council of Agricultural Research, India, under whose employment the author has carried out this work, and to the University of Calcutta for permission to work in the Physical Chemistry Laboratories of the University College of Science and for other facilities.

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Received September 23, 1939.

THE ELECTROCHEMICAL PROPERTIES OF COLLOIDAL SILICIC ACID. PART II. INTERACTION WITH NEUTRAL SALTS.*

BY B. CHATTERJEE.

The capacities of different cations to liberate acid from colloidal silicic acid are in the order $\text{Ba}^{++} > \text{Ca}^{++} > \text{Na}^+ > \text{Li}^+$, i.e., in agreement with the lyotrope series. The total amount of acid liberated by BaCl_2 , $\text{Ba}(\text{CH}_3\text{COO})_2$, CaCl_2 and $\text{Ca}(\text{CH}_3\text{COO})_2$ is considerably greater than that neutralised at the first inflexion point in the titration curve of silicic acid sol with a dilute base. At low concentrations, alkali metal cations only effect an osmotic displacement of the mobile H^+ ions. The results have been discussed in the light of the theory of electrical double layer and secondary adsorption of ions.

The nature of the interaction between neutral salts and colloidal silicic acid has been the subject of controversies. Joseph and Hancock (*J. Chem. Soc.*, 1923, 128, 202) regarded the reaction as an ordinary double decomposition process in which insoluble silicates are formed with the liberation of acids, the equilibrium condition being determined by the solubility of the solid salts (silicates formed by the interaction) and of silicic acid. Mukherjee and co-workers (*J. Chem. Soc.* 1926, 3023), on the other hand, considered that the reaction is not a simple chemical process but consists of interchanges between the hydrogen ions present in the double layers associated with the colloidal particles (Mukherjee, *Trans. Faraday Soc.*, 1921, 16, 103; *Phil. Mag.*, 1922, 44, 321) of the sol and the cations of the added salts. According to them the amount of hydrogen ions exchanged at equilibrium is, therefore, determined by the relative adsorbabilities of the cations of the salts rather than by the solubilities of the corresponding solid salts. It follows from the theory of electrical adsorption of oppositely charged ions by charged colloidal surface postulated by Mukherjee (*Trans. Faraday Soc.*, 1921, 16, 103; *Phil. Mag.*, 1922, 44, 321; *Kolloid Z.*, 1939, 62, 257) that the energy of electrical adsorption of barium ions is greater than that of calcium ions. Barium chloride should, therefore, on interaction with the sol, liberate more acid than calcium chloride. Solubility considerations, however, lead one to expect a greater relative effect of calcium chloride because of the greater insolubility of calcium silicate. These conflicting inferences from the rival theories can be put to experimental test. Mukherjee and co-workers (*loc. cit.*) observed that the supernatant liquid of purified silica gel showed

* The results given in this paper have been taken from the annual reports (1936-37, 1937-38, 1938-39) submitted to the Imperial Council of Agricultural Research, India.

a greater concentration of H^+ -ions on the addition of barium chloride than calcium chloride; an observation which supports their theory. They, however, measured the H^+ -ion concentration by the indicator method. The limitations of this method for measurements with unbuffered acid system are well known. It is also necessary to have more complete evidence which should preferably be confirmed by different methods.

In this paper a careful study has been made by comparing (1) the variations in the H^+ -ion concentration of silicic acid sols on the progressive additions of solutions of neutral salts and (2) the total amount of acid liberated on continually leaching the sols with these solutions till the leachates show a neutral reaction. The hydrogen electrode and in some cases the glass electrode have been used for the measurements. Other salts have also been used. The experimental arrangements were the same as those described in Part I (p. 583).

Variation in the p_H values of Silicic Acid Sols on the Addition of Neutral Salt Solutions.

From the titration curves given in Figures 1, 2 and 3 and the results given in Table I it will be seen that (1) potassium chloride has a smaller effect on the lowering of p_H of the sols than barium chloride or calcium chloride and (ii) the p_H decreases rapidly in the initial stages of the titrations but the rate of decrease diminishes with the gradual additions of the salt solutions.

TABLE I.

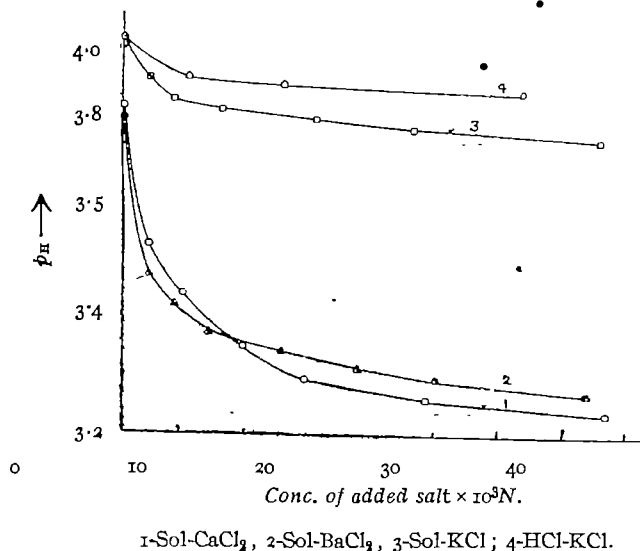
Sol.	Salt conc.	Lowering of the p_H with $BaCl_2$.	Lowering of the p_H with $CaCl_2$.	Sol.	Salt conc.	Lowering of the p_H with $BaCl_2$.	Lowering of the p_H with $CaCl_2$.
L'	0			N	0		
	$2.0 \times 10^3 N$	0.20	0.14		$2.0 \times 10^3 N$	0.19	0.07
	4.0	0.23	0.14		5.0	0.24	0.13
	7.0	0.23	0.13		10.0	0.26	0.13
	13.0	0.21	0.12		20.0	0.26	0.13
	20.0	0.20	0.13		40.0	0.25	0.12
	32.5	0.22	0.13		55.0	0.28	0.12
M	0			P	0		
	2.0	0.24	0.17		2.0	0.27	0.14
	5.0	0.23	0.20		3.0	0.30	0.18
	10.0	0.26	0.22		5.0	0.32	0.21
	20.0	0.29	0.25		10.0	0.31	0.22
	40.0	0.34	0.25		20.0	0.31	0.23
	80.0	0.39	0.28		40.0	0.32	0.22
					80.0	0.34	0.22

The changes in the p_H may be ascribed at least in part, to changes in the liquid junction potential and in the H^+ -ion activities and therefore require to be corrected. Control measurements were therefore carried out with hydrochloric acid having nearly the same p_H as the sol. The lowering of p_H observed with the sols has been corrected for that observed with HCl in the actual measurements and the corrected data are given in Table I.

A definitely greater lowering of p_H on addition of barium chloride than calcium chloride is evident. The greater effect of barium is in agreement

FIG. 1.

Sol K.

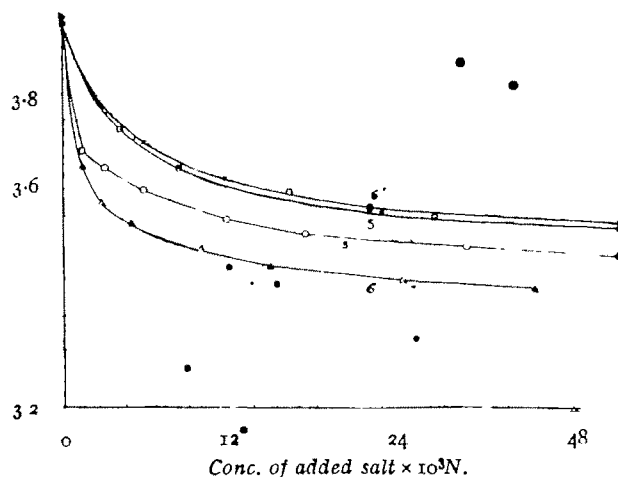


with the theory of electrical adsorption. Calcium hydroxide, however, reacts more strongly with silicic acid sol than baryta. (*vide* Part I, *This Journal*, p. 583).

Curves 1 and 2 in Fig. 1 obtained with sol K are interesting as they bring out a factor which sometimes obscures the comparison. It will be seen that barium chloride lowers the p_H to a greater extent in the initial stages but at concentrations higher than 0.0085*N*-calcium chloride shows a greater effect. But sol K was found to have set to a gel in presence of 0.0085*N*-barium chloride but not of calcium chloride. The setting renders difficult an intimate mixing of the electrolyte with the sol.

FIG. 2.

Sol L'

5-Sol-CaCl₂; 6 Sol-BaCl₂; 5'-HCl-CaCl₂; 6'-HCl-BaCl₂

*Estimation of the Total Amount of Acid liberated by continually
Leaching the Sol with Solutions of BaCl₂ and CaCl₂.*

In comparing the relative effects of barium and calcium chloride an estimation of the total acidity of the sols in the presence of these salts would be more helpful and decisive. The sols, however, set on trying to titrate them with bases in the presence of barium or calcium chloride. The amount

TABLE II.

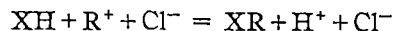
Sol	Amount of acid liberated in normality			
	BaCl ₂ .	CaCl ₂	NaCl.	*LiCl
M 1st leachate	16.0 × 10 ⁻⁵	11.4 × 10 ⁻⁵		
2nd	5.8	5.4		
3rd	3.6	3.4		
4th	Nil	Nil		
Total	25.4	20.2		
Q 1st leachate	17.0	15.0	11.8 × 10 ⁻⁵	9.8 × 10 ⁻⁵
2nd	8.4	4.0	4.0	4.6
3rd	3.8	2.6	3.0	1.0
4th	Nil	Nil	Nil	Nil
Total	29.2	21.6	18.8	15.4

* 0.013N solution of LiCl has been used for leaching sol Q

of acid liberated from the sol by continually leaching it with barium or calcium chloride has, therefore, been estimated. The total amounts of acid liberated by NaCl and LiCl from sol Q have also been estimated and incorporated in Table II for comparison. Normal solutions of neutral salts have been used for sol M and centinormal solutions of neutral salts for sol Q.

The amount of acid liberated by the salts are considerably greater, specially by BaCl₂ and CaCl₂ than that neutralised at the first inflexion point in the titration curve of sols with dilute bases ($9.0 \times 10^{-6}N$ and $14.5 \times 10^{-5}N$ for sols M and Q respectively). It will be seen that BaCl₂ and CaCl₂, on account of their greater electrical adsorption, have displaced greater amount of acid than that liberated by NaCl or LiCl. The capacities of the different cations to liberate acid are in the order $Ba^{++} > Ca^{++} > Na^{+} > Li^{+}$. The results also confirm beyond doubt the greater relative effect of barium than calcium ions.

The reaction between the sol and the salts (here, chlorides) is really a balanced one and can be represented as follows :



where H stand for the 'mobile' as well as the 'bound' H⁺-ions present in the double layer and X represents the colloidal anion. With the progress of the reaction there will be an increase in the concentration of hydrochloric acid, which being highly dissociated, the H⁺-ion concentration will greatly increase and consequently the backward reaction will be favoured more and more. The displacement of H⁺-ions from the double layer by the cations of the added salt will be rendered more and more difficult. In view of the weaker dissociation of acetic acid it was thought that the displacement of the H⁺-ions would be more complete if acetates instead of chlorides were used. The results obtained with sol Q are given in Table III. Centinormal solutions of neutral salts have been used.

TABLE III.

	Total acid	
	(CH ₃ COO) ₂ Ba.	(CH ₃ COO) ₂ Ca.
1st leachate	$32.5 \times 10^{-5}N$	$38.0 \times 10^{-5}N$
2nd	22.5	5.0
3rd	11.5	Nil
4th	3.5	—
Total	70.0	43.0

As expected the amount of acid liberated by barium and calcium acetates is considerably greater than that liberated by the corresponding chlorides. Barium acetate again liberates more acid than calcium acetate,

The results show that the amount of acid liberated is highest in the case of $\text{Ba}(\text{CH}_3\text{COO})_2$. Even then, the total amount of acid liberated by $\text{Ba}(\text{CH}_3\text{COO})_2$ is only 4.5 milliequivalents per 100 g. of colloid. This value is extremely low in comparison with that observed in the case of most hydrogen clays or bentonites.

According to Mukherjee's theory, the solid surface of the colloidal particles of silicic acid carries anions constituting the fixed layer of primarily adsorbed anions, some kind of silicate ions. An equivalent amount of hydrogen ions held on or near the surface satisfies the condition of electroneutrality of the system. A part of these hydrogen ions remains fixed on the surface by electrostatic or chemical forces and these constitute what he terms the secondarily adsorbed layer, while the rest of the hydrogen ions forms the mobile sheet of the double layer. These mobile hydrogen ions register their activities on a hydrogen electrode and give rise to the observed p_H of the sol (Mukherjee, *loc. cit.*).

The first process, *viz.*, the displacement of the mobile hydrogen ions, is the result of osmotic diffusion and should not materially alter the hydrogen ion activity of the sol as the total number of osmotically active hydrogen ions in the system does not change. The second process, namely, the displacement of bound or secondarily adsorbed hydrogen ions should increase the hydrogen ion activity of the systems. The displacement of bound hydrogen ions depends on the adsorbabilities of the cations and is determined by their valencies and mobilities, when the potential energy of resulting ion pairs is purely the result of electrostatic attractive forces between the primarily adsorbed ion and the hydrated cation constituting them.

In the following section the existence or otherwise of the first process has been examined. The existence of the second process follows from the data in Tables I, II and III.

Displacement of Mobile Hydrogen Ions by the Cation of the Added Salt.

The weak electrical adsorption of alkali metal ions renders it possible that in low concentrations they would not be capable of displacing to any large extent the bound hydrogen ions and would produce only an osmotic displacement of mobile hydrogen ions from the double layer to the bulk phase. Consequently there will be no appreciable change in the p_H of the sol.

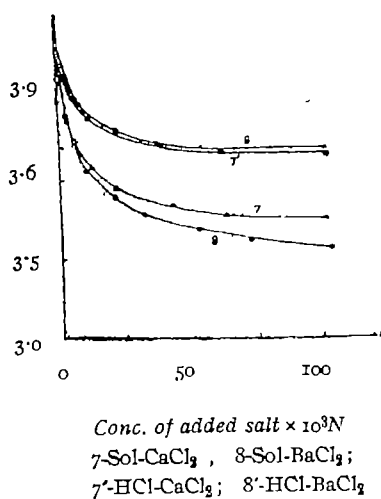
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The p_H variation on addition of KCl at low concentrations to the sol K are given below

TABLE IV.

	p_H		Average.	*Total acidity.
	Hydrogen electrode.	Glass electrode.		
Sol K	4.00 3.92 3.98	3.97 4.02 4.00	3.98	$10.00 \times 10^{-5}N$
Ultrafiltrate of sol K	—	5.09		1.1
Sol K + 0.01N-KCl	3.80 3.91	3.87 3.87	3.86	
Ultrafiltrate of above mixture	4.10	4.16	4.13	11.4

FIG. 3.



The above results show that (i) the total acidity of the ultrafiltrate of the sol+salt mixture is practically equal to that of the sol, though the total acidity of the ultrafiltrate of the sol itself is only about 10% of that of the sol; (ii) the addition of 0.01N-KCl produces a change in the p_H of the sol from 3.98 to 3.86 owing mainly to the changes in the liquid junction potential referred to earlier (*vide* curves 3 and 4), (iii) the addition of KCl,

however, has brought down the p_H of the ultrafiltrate from 5.09 to 4.13, the latter p_H is almost equal to that of the sol and the sol + salt mixture, showing that the mobile hydrogen ions, which could not originally pass through the ultrafilter membrane, can do so on the addition of KCl at the

* Total acidity values have been calculated from the inflexion points in the titration curves with dilute bases.

TABLE V.

	p_H			Free acidity.	Total acidity
	Hydrogen electrode	Quinhydrone electrode	Average		
Sol Q	4.09	4.12	4.11	$7.8 \times 10^{-5}N$	$14.5 \times 10^{-6}N$
	4.10				
	4.10				
Ultrafiltrate of above	5.02	5.07	5.05	0.89	1.2
Sol Q 0.01N-LiCl	4.12	4.10	4.11	7.8	*
	4.12				
Ultrafiltrate of the above	4.14				
	4.08	4.08	4.11	7.8	7.4
	4.07	4.15			8.0
	4.10				8.2
				Average	7.9

above concentrations. Similar results with lithium chloride using sol Q are shown in Table V.

As in the previous case, the free acidities of the sol, the sol+salt mixture and of the ultrafiltrate of the mixture are in mutual agreement indicating a complete displacement of the mobile hydrogen ions by the lithium ions. The total acid of the ultrafiltrate is also in fair agreement with its free acid. This is to be expected as the ultrafiltrate should contain only hydrochloric acid. The total acid of the sol, on the other hand, is considerably higher than its free acid showing that the inflexion point in the titration curve from which this total acidity has been calculated involves the neutralisation of some bound H^+ -ions in addition to the mobile hydrogen ions initially present in the sol.

It is intended to study in further detail the interaction between colloidal silicic acid and cations of the alkali and alkaline earth metals.

CONCLUSION.

The p_H of a silicic acid sol is considerably lowered on the addition of neutral salts. The effect of alkali metal cations is much smaller than that

* Could not be determined as the mixture set on the addition of alkali.

of the alkaline earth ones. The Ba^{++} -ions are more effective than the Ca^{++} -ions.

The total amount of acid liberated by continually leaching a silicic acid sol with neutral solutions of BaCl_2 , CaCl_2 , $\text{Ba}(\text{CH}_3\text{COO})_2$ and $\text{Ca}(\text{CH}_3\text{COO})_2$ is considerably greater than that neutralised at the first inflexion point in the titration curve of the sol with a dilute base. Ba- and Ca-acetates liberate more acid than the corresponding chlorides. The total amount of acid liberated by Ba-salts is much greater than that liberated by Ca-salts. The capacities of different cations to liberate acid from silicic acid sol are in the order : $\text{Ba}^{++} > \text{Ca}^{++} > \text{Na}^+ > \text{Li}^+$.

At low concentrations the alkali metal cations on account of their weak electrical adsorption can not easily displace the 'bound' H^+ -ions but only effect an osmotic displacement of the 'mobile' hydrogen ions.

The results have been discussed in the light of the theory of electrical double layer and of the secondary adsorption of ions.

The author takes this opportunity to offer his thanks to Prof. J. N. Mukherjee, D.Sc, for his suggestions and advice, to the Imperial Council of Agricultural Research, India, under whose employment the author has carried out this work and to the University of Calcutta for permission to work in the Physical Chemistry Laboratories of the University College of Science and for other facilities

PHYSICAL CHEMISTRY LABORATORY,
UNIVERSITY COLLEGE OF SCIENCE
AND TECHNOLOGY, CALCUTTA

Received September 23, 1939

COMPLEX COMPOUNDS OF BIGUANIDE WITH BIVALENT METALS. PART I. COPPER BIGUANIDINES.

BY PRIYADARANJAN RÂY AND PHANINDRA NATH BAGCHI.

The free anhydrous cupric biguanidine has been obtained from the hydrated variety. This furnishes an additional evidence in support of the constitution of these complexes, previously suggested. It has been further shown that the formation of complexes with biguanide confers great stability on many unstable simple cupric salts like iodide sulphite, thiosulphate, thiocyanate and hypophosphite. Besides these, a number of other new cupric bisbiguanidinium salts, such as fluoride, bromide, nitrite, carbonate, dithionate and chromate, has been described.

Certain complex compounds of biguanide and its substitution products with bivalent metals like copper, nickel and cobalt have been described from very early times. Rathke (*Ber.*, 1879, 12, 779) prepared simple copper biguanide sulphate and formulated it as $(C_2N_3H_6)_2Cu \cdot H_2SO_4$. Emich (*Monatsh.*, 1883, 4, 395) prepared copper derivatives of ethylbiguanide sulphate by the action of copper sulphate on dicyandiamide dissolved in ethylamine. Smolka and Friedrich (*ibid.*, 1888, 9, 227) subsequently isolated phenylbiguanide derivatives of cobalt, copper and nickel. Rudolf Ziegelbauer (*Monatsh.*, 1896, 17, 648) prepared *ortho*-phenylenebiguanide derivatives of cobalt and nickel (*cf.* Dubskey, Langer and Strand, *Collection*, 1938, 10, 112). Finally R. Andreasch (*Monatsh.*, 1927, 48, 145) prepared copper biguanide hydrochloride and nitrate by the addition of biguanide chloride and nitrate on ammoniacal copper chloride and nitrate respectively. With each atom of the bivalent metal two molecules of biguanide were found to be associated. The constitution of complex compounds of biguanide with bivalent metals has already been discussed by Rây and Saha (*J. Indian Chem. Soc.*, 1937, 14, 15).

In the present paper a number of complex copper biguanide salts has been described besides the free hydrated and anhydrous base. Co-ordination with the biguanide molecule has been found to confer stability to many unstable simple salts of copper, such as the iodide, sulphite, thiocyanate, hypophosphite and thiosulphate, which, as is well known, readily change into the cuprous state under ordinary circumstances. Besides these, fluoride, chloride, bromide, dithionate, chromate, carbonate, nitrate and nitrite of the complex copper biguanide base have been prepared and their properties studied. Of these the copper biguanide hydrate, chloride, nitrate and sulphate, as already stated, have been described by previous workers.

EXPERIMENTAL.

Cupric bisbiguanide dihydrate was prepared in the form of bright red silky plates from ammoniacal copper sulphate solution and an alkaline solution of biguanide sulphate as described by Herth (*Ber.*, 1880, 13, 1358). The substance was dried over caustic potash to a constant weight. {Found : N, 46.77; Cu, 21.31; H₂O (by loss at 110°), 11.95. [Cu(Big)₂]·2H₂O requires N, 46.73; Cu, 21.22; H₂O, 12.01 per cent} where BigH = one biguanide molecule.

The substance readily absorbs carbon dioxide from air, liberates ammonia from hot ammonium chloride solution and combines with two equivalents of hydrochloric acid to form the chloride. It thus behaves as a diacidic base.

Cupric bisBiguanidine—When the dihydrate was heated to 110° for 14 hours it lost the whole of its water. {Found : N, 52.93; Cu, 24.0. [Cu(Big)₂] requires N, 53.11; Cu, 24.12 per cent}.

Cupric bisBiguanidinium Chloride.—When the powdered base was heated with 15% ammonium chloride solution to 60°-70° for some time on the water-bath, it dissolved with evolution of ammonia. The solution was filtered hot and on cooling pure crystals of the chloride separated from the filtrate. These were washed first with ice-cold water, then with alcohol and finally dried in air. The salt can also be prepared by treating the base with dilute hydrochloric acid. The substance forms red needle-shaped crystals, readily soluble in water. It decomposes on treatment with dilute acids. The same salt was prepared also by R. Andreasch (*loc. cit.*) from ammoniacal copper chloride solution and biguanide hydrochloride. {Found : N, 37.44; Cl, 19.18; Cu, 17.02; H₂O (by loss at 105°), 9.59. [X] Cl₂·2H₂O requires N, 37.57; Cl, 19.06; Cu, 17.07; H₂O, 9.66 per cent} where X = [Cu(BigH⁺)₂].

Equivalent Conductivity at 35°.

<i>v</i> (litres)	16	32	64	128	256	512	1024
λ_v ...	104.6	115.2	127.5	129.0	131.6	135.3	141.6
λ_∞ ...	140.8	143.1	149.6	144.7	142.9	143.5	147.7

(from Walden's formula)

λ_∞ (mean) = 144.6

Therefore, the mobility of $1/2$ [Cu(BigH⁺)₂] = 144.6 - 66.7 = 77.9, the mobility of chlorine ion being 66.7.

Cryoscopic Measurement.

Subs./100 g. of water (on the anhydrous basis .	Depression in °C Δ	Mol. wt m .	van't Hoff's factor $i = M/m$.	Degree of dis- sociation. $\alpha = i - 1/n - 1$
0.5483	0.083	119.0	2.83	0.91
0.2741	0.041	120.3	2.80	0.90

The result shows that even at 0° the substance is dissociated almost completely into three ions and the whole of the chlorine is in the ionic state.

When heated to 105° for 4 hours, the salt lost the whole of its water. The anhydrous chloride gave {Found: Cl, 20.86; Cu, 18.82. $[\text{Cu}(\text{BigH}^+)_2]\text{Cl}_2$ requires Cl, 21.09; Cu, 18.89 per cent}. The bluish violet anhydrous chloride readily absorbs water to regenerate the hydrated compound.

Cupric bisbiguanidinium bromide was obtained by heating the base with chemically pure ammonium bromide on the water-bath at about 80° until a clear solution resulted. The solution was filtered hot by suction. The crystals obtained by cooling the filtrate were filtered, washed and dried as described above. It forms brick-red needle-shaped crystals and resembles the chloride in properties. {Found: N, 30.24; Br, 34.58; Cu, 13.75. $[\text{X}]\text{Br}_2 \cdot 2\text{H}_2\text{O}$ requires N, 30.34; Br, 34.63; Cu, 13.78 per cent}.

Cupric bisbiguanidinium iodide was prepared from the complex base and ammonium iodide as described in the previous case. The substance forms red needle-shaped crystals much less soluble in water than the other halides. {Found: N, 24.52; I, 44.39; Cu, 11.18. $[\text{X}]\text{I}_2 \cdot 3\text{H}_2\text{O}$ requires N, 24.40; I, 44.28; Cu, 11.08 per cent}.

Cupric bisbiguanidinium fluoride was prepared like the previous compounds from the base and the neutral ammonium fluoride. It is more soluble than the other halides and forms red needle-shaped crystals. {Found: N, 37.35; F, 10.24; Cu, 17.06. $[\text{X}]\text{F}_2 \cdot 4\text{H}_2\text{O}$ requires N, 37.27; F, 10.11; Cu, 16.93 per cent}.

Cupric bisbiguanidinium nitrate, as in the case of previous compounds, was obtained from the base and ammonium nitrate in the form of pale rose crystals, sparingly soluble in cold water. It can also be prepared by adding a solution of ammonium nitrate to a concentrated solution of the chloride. The same substance was also prepared by Andreasch (*loc. cit.*) from ammoniacal copper nitrate solution and biguanide nitrate. {Found: N (total), 39.68; Cu, 15.03; NO_3 , 29.27. $[\text{X}](\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ requires N, 39.47; Cu, 14.94; NO_3 , 29.13 per cent}.

Cupric bisbiguanidinium nitrite was prepared by adding a solution of sodium nitrite to a concentrated solution of the chloride. The crystals were washed and dried as usual. It resembles the nitrate in colour and, like the latter, is sparingly soluble in water. {Found: N(total), 45.17; Cu, 16.79;

NO_2 , 24.37. [X] $(\text{NO}_2)_2 \cdot \text{H}_2\text{O}$ requires N, 44.72; Cu, 16.93; NO_2 , 24.49 per cent}.

Cupric bisbiguanidinium carbonate was precipitated by adding a solution of sodium carbonate to a concentrated solution of the chloride. {Found: N, 35.02; Cu, 16.07; CO_3 , 15.33. [X] $\text{CO}_3 \cdot 4\text{H}_2\text{O}$ requires N, 35.11; Cu, 15.99; CO_3 , 15.09 per cent}.

Cupric bisbiguanidinium sulphite was prepared by adding, drop by drop, a cold concentrated, freshly prepared solution of sodium sulphite to a cold saturated solution of the chloride. It forms rose-red crystals, sparingly soluble in water. {Found: N, 33.29; S, 7.54; Cu, 15.11. [X] $\text{SO}_3 \cdot 4\text{H}_2\text{O}$ requires N, 33.52; S, 7.66; Cu, 15.23 per cent}.

Cupric bisbiguanidinium thiosulphate was obtained as a pale rose crystalline precipitate by adding a solution of sodium thiosulphate to a concentrated solution of the chloride. {Found: N, 32.65; S, 14.70; Cu, 14.63. [X] $\text{S}_2\text{O}_3 \cdot 3\text{H}_2\text{O}$ requires N, 32.43; S, 14.82; Cu, 14.73 per cent}.

Cupric bisbiguanidinium thiocyanate was prepared by heating the copper biguanide base with a solution of ammonium thiocyanate. It forms shining bluish violet crystals, sparingly soluble in water. {Found: S, 16.65; Cu, 16.63. [X] $(\text{SCN})_2$ requires S, 16.77; Cu, 16.66 per cent}.

Cupric bisbiguanidinium dithionate was prepared by adding a solution of sodium dithionate to a concentrated solution of the chloride. It forms rose-red crystals, soluble in water. {Found: S, 13.72; Cu, 13.88. [X] $\text{S}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$ requires S, 13.86; Cu, 13.77 per cent}.

Cupric bisbiguanidinium chromate was obtained as a yellow crystalline precipitate from a solution of potassium chromate and the complex chloride. {Found: N, 31.96; Cr, 11.79; Cu, 14.52. [X] $\text{CrO}_4 \cdot 3\text{H}_2\text{O}$ requires N, 32.13; Cr, 11.93; Cu, 14.60 per cent}.

Cupric bisbiguanidinium hypophosphite was precipitated as rose-red crystals by adding a solution of sodium hypophosphite to a concentrated solution of the chloride. {Found: Cu, 14.85; P, 14.42. [X] $(\text{H}_2\text{PO}_2)_2 \cdot 2\text{H}_2\text{O}$ requires Cu, 14.73; P, 14.36 per cent}.

A solution of the complex chloride gave pale rose, moderately soluble, crystalline precipitate with potassium chlorate, bromate and iodate. Sparingly soluble pale rose precipitates were also obtained with alkali perchlorate and periodate. It also gave characteristic coloured precipitates with a number of complex anions, *e.g.* ferrocyanide, ferricyanide, cobalticyanide, nitroprusside, thiosulphato-cobalticyanide, disulphito-cobalticyanide, chromithiocyanate, cobaltinitrite, borofluoride, aurichloride, platinichloride, etc.

COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS. PART VI. COBALTIC TRISBIGUANIDINES.

BY PRIYADARANJAN RÂY AND NIHAR KUMAR DUTT.

Tervalent cobalt has been found to combine with biguanide to form complex cobaltic *trisbiguanidine*, its hydrate and a series of well-defined salts, resembling the corresponding chromium complex and its derivatives in composition and properties.

With the exception of a cobaltic *ortho*-phenylenebiguanide compound (Ziegelbauer, *Monatsh.*, 1896, **17**, 648; Dubsky, Langer and Strand, *Collection*, 1938, **10**, 112), no other complex biguanide compound of tervalent cobalt, either with simple or substituted biguanide, has been described in literature. In previous communications of this series (Rây and Saha, *J. Indian Chem. Soc.*, 1937, **14**, 670; 1938, **15**, 353, 633; Rây and Ghosh, *ibid.*, 1938, **15**, 347, 350) complex compounds of tervalent chromium with simple and phenyl biguanide have been described and the constitution of the metallic biguanide complexes in general has been discussed. Study of the complex compounds of tervalent cobalt with simple biguanide—cobaltic *trisbiguanidines*—forms the subject matter of this communication.

Cobaltic *trisbiguanide* complexes closely resemble the corresponding chromium compounds in their composition and also in their physical and chemical properties. Like chromium, cobalt also forms an anhydrous *trisbiguanidine*, but gives rise to a solid dihydrated base, while the corresponding chromium compound is monohydrated. In solution both behave as triacidic base corresponding to the composition of their salts. The colour of cobaltic *trisbiguanide* compounds simulates those of its chromium analogues, except that the latter are more brightly coloured. The solubility of the two series of compounds runs in a parallel course, the chromium compounds being comparatively less soluble. The cobaltic biguanide complexes are, however, relatively much more stable than their chromium counterparts, and do not hydrolyse like the latter to form *bisbiguanide* derivatives in any appreciable amount even at the boiling temperature in solution.

The constitution of the cobaltic *trisbiguanide* complexes may be represented like those of chromium by the formula $[\text{Co}(\text{BigH}^+)_3] \text{X}_3$, where $\text{X}=\text{OH}$ or any univalent or its equivalent acid radicle. The anions, as in the case of chromium, are derived from a secondary co-ordination by the free amino group at one end of each biguanide molecule, the amino group at the other end co-ordinating with the central cobalt atom. The hydrogen atom of one of the outer imino ($=\text{NH}$) groups in each biguanide molecule is replaced by the cobalt atom through the exercise of

its primary valency. In other words, the central cobalt atom, as already discussed in the case of chromium complexes (Rây and Saha, *loc. cit.*), forms a neutral inner metallic complex by co-ordinating with three bidentate biguanide molecules, the cationic character of which is due solely to the free amino groups of the latter

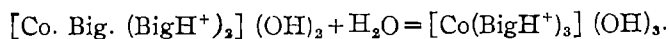
The preparation and properties of the anhydrous cobaltic *trisbiguanidine*, its dihydrate and a series of salts in which the complex behaves as a triacidic base are described in this paper.

The nature of its constitution as a hexa-co-ordinated complex with three bidentate molecules suggests at once the possibility of resolution into optically active modifications. In the case of chromium *trisbiguanide* compounds attempts at such resolution failed due to their more or less rapid hydrolysis into *bisbiguanide* derivatives. The stability of the cobaltic *trisbiguanide* complexes presents, however, a very favourable case for such resolution, and we have actually succeeded in preparing both the *dextro*- and *laevo*-series of these complexes. These will be reported in our next communication.

EXPERIMENTAL.

Cobaltic trisBiguanide Dihydrate.—A solution of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (5g.) was added to a solution of biguanide sulphate (17 g.) in strong alkali, when the yellow cobaltous biguanide was precipitated. This was oxidised by passing a vigorous current of air for 5-6 hours into the alkaline suspension. More alkali solution was added from time to time when the whole mass turned deep red with the formation of cobaltic biguanide in quantities. The mixture was then cooled and filtered on the pump. The crystals were purified by recrystallisation from slightly alkaline hot water. These were then dried in air, free from CO_2 . {Found : N, 53.0, 53.22; Co, 14.97. $[\text{Co}(\text{Big})_3] \cdot 2\text{H}_2\text{O}$ requires N, 53.16; Co, 14.93 per cent} where $\text{BigH} = \text{one biguanide molecule}$.

The substance forms beautiful dark red crystals, which are moderately soluble in water giving a strongly alkaline solution. It liberates ammonia from ammonium salts, precipitates hydroxides of heavy metals from solutions of their salts, and behaves as a triacidic base in aqueous solution.



0.4056 G. of the substance in aqueous solution required 6.95 c.c. of 0.451N- H_2SO_4 for neutralisation. $[\text{Co}(\text{BigH}^+)_3] (\text{OH})_3$ requires 6.85 c.c.

Measurement of the equivalent conductivity and the determination of the freezing point of the solution of the base also lead to the same conclusion.

Equivalent Conductivity at 25°.

<i>v</i> (dilution) ...	16	32	64	128	256	512	1024
λ_v ...	25.9	33.9	44.2	55.4	66.6	76.6	92.0

• *Cryoscopic Measurement.*

Subs. (g./100 c.c.)	Depression. Δ	Mol. wt <i>m</i>	van't Hoff's factor $i = M/m$	Degree of dissociation. $\alpha = i - 1/n - 1$
0.2671	0.041°	114.5	3.6	0.87

Where M = mol. wt. calculated as trihydrated base, n = no. of ions = 4.

The above results indicate that the substance is as strong a base as the alkali metal hydroxides.

Definite evidence regarding the triacidic nature of the base is, however, furnished by the preparation of a series of crystalline salts as described hereafter, in which one molecule of the base is found to combine with three equivalents of the acid. The determination of the valency of the complex ion by means of Ostwald-Walden's rule from the measurement of the equivalent conductivity of its hydrochloride furnishes additional proof thereto.

Cobaltic trisBiguanidine.—The dihydrate described above, when heated to 120–122° for some time, lost 9.057% of its weight. Percentage of water in the dihydrate is 9.1. The substance decomposes at higher temperatures. {Found: N, 58.20. $[\text{Co}(\text{Big})_3]$ requires N, 58.49 per cent}. The anhydrous base is dull red in colour and readily absorbs water.

Cobaltic trisbiguanidinium chloride was prepared by treating the solid base in a mortar with ice-cold dilute hydrochloric acid till the mixture gave a slightly acid reaction. The yellow crystals formed, were filtered and washed with a little ice-cold water. These were purified by recrystallisation from hot water. The crystals were washed first with ice-cold water, then with absolute alcohol and afterwards dried in air. The yellow crystals of the chloride are readily soluble in water. {Found: N, 45.03; Cl, 23.0, 23.12; Co, 12.69, 12.60. $[\text{Co}(\text{BigH}^+)_3]\text{Cl}_3$ requires N, 44.85; Cl, 22.88; Co, 12.67 per cent}.

Equivalent Conductivity at 25°.

<i>v</i> (dilution) ...	16	32	64	128	256	512	1024
λ_v ..	90.7	101.1	110.0	116.8	123.3	126.5	130.6
λ_∞ ..	138.0	138.0	138.5	138.0	139.0	138.0	139.0

Mean $\lambda_\infty = 138.5$.

The λ_{∞} was calculated from Walden's formula,

$\lambda_{\infty} = \lambda_v (1 + n_1 n_2 \times 0.692 v^{-\frac{1}{2}})$; where v = dilution, n_1 and n_2 are the valencies of the ions.

The valency of the complex ion according to Ostwald-Walden's rule is given by $\lambda_{102.4} - \lambda_{3.2} = 130.6 - 101.1 = 29.5 = 10 \times 2.95$. The valency is, therefore, three.

Cryoscopic Measurement.

Determination of the molecular weight by the freezing point method leads also to the same conclusion.

Subs. (g./100 c.c.).	Depression. Δ	Mol. wt m .	van't Hoff's factor. $i = M/m$	Degree of dissociation $\alpha = i - 1/n - 1$.
0.5784	0.088°	118.3	3.96	0.98

The result shows that even at 0° the substance dissociates almost completely into four ions and all the chlorine are present as ions.

Cobaltic trisbiguanidinium fluoride was obtained as soluble yellow crystals by adding a concentrated solution of ammonium fluoride to a strongly cooled, concentrated solution of the chloride. The crystals were washed and dried as described above. {Found: F, 13.31; Co, 14.0. [Co(BigH⁺)₃] F₃ requires F, 13.70; Co, 14.18 per cent}.

Cobaltic trisbiguanidinium bromide was obtained in the form of bright yellow crystals by precipitating a solution of the chloride with a strong solution of potassium bromide {Found: Br, 40.05; Co, 9.88. [Co(BigH⁺)₃] Br₃ requires Br, 40.07; Co, 9.83 per cent}.

Cobaltic trisbiguanidinium iodide was obtained as yellow crystals from chloride and potassium iodide. It is less soluble than the other halides. {Found: I, 51.13; Co, 8.0. [Co(BigH⁺)₃] I₃ requires I, 51.48; Co, 7.97 per cent}.

Cobaltic trisbiguanidinium thiocyanate was obtained in the form of reddish yellow crystals, soluble in water, by the double decomposition of the complex chloride and ammonium thiocyanate. It can also be prepared by heating a strong solution of the complex base with ammonium thiocyanate. {Found: S, 18.02; Co, 11.07. [Co(BigH⁺)₃](SCN)₃ requires S, 17.91; Co, 11.07 per cent}.

Cobaltic trisbiguanidinium chlorate was prepared by precipitating a strong solution of the complex chloride with a saturated solution of potassium chlorate. It forms yellow crystals, moderately soluble in water. {Found: Cl, 17.40; Co, 9.59. [Co(BigH⁺)₃](ClO₃)₃ requires Cl, 17.47; Co, 9.68 per cent}.

Cobaltic trisBiguanidinium Perchlorate.—The readily soluble, orange-yellow crystals of the perchlorate were obtained by treating the finely powdered solid base in a mortar with a cold strong solution of perchloric acid. The crystals were washed and dried as usual. {Found: Cl, 16.10, Co, 8.98. $[\text{Co}(\text{BigH}^+)_3](\text{ClO}_4)_3$ requires Cl, 16.19; Co, 8.98 per cent}.

Cobaltic trisbiguanidinium borofluoride was prepared by heating a strong solution of the complex base with a concentrated solution of ammonium borofluoride till there was no further evolution of ammonia. The substance forms reddish yellow crystals, soluble in water. {Found: Co, 9.39. $[\text{Co}(\text{BigH}^+)_3](\text{BF}_4)_3$ requires Co, 9.51 per cent}.

Cobaltic trisbiguanidinium nitrate was obtained as orange-yellow crystals by neutralising the base with cold dilute nitric acid. The crystals were washed at first with ice-cold water and then with alcohol. These were afterwards dried in air. The crystals are readily soluble in water. {Found: Co, 10.65; NO_3 , 34.01. $[\text{Co}(\text{BigH}^+)_3](\text{NO}_3)_3$ requires Co, 10.83; NO_3 , 34.13 per cent}.

Cobaltic trisbiguanidinium nitrite was prepared by precipitating a strong solution of the complex chloride with a cold concentrated solution of potassium nitrite. It forms orange-yellow crystals, soluble in water. {Found: N (nitritic), 8.41; Co, 11.80. $[\text{Co}(\text{BigH}^+)_3](\text{NO}_2)_3$ requires N (nitritic), 8.45; Co, 11.87 per cent}.

Cobaltic trisBiguanidinium Chloro-carbonate.—When a strong solution of sodium carbonate was added to a cold concentrated solution of the complex chloride, the orange-yellow crystals of the chloro-carbonate separated from the solution. The mixture was cooled in ice and the crystals that finally separated were washed and dried as usual. The substance is readily soluble in water, the solution being strongly alkaline to litmus. {Found: C, 16.80; Cl, 15.20; Co, 12.60. $[\text{Co}(\text{BigH}^+)_3]_2\frac{\text{CO}_3}{\text{Cl}_4}$ requires C, 16.85; Cl, 15.43; Co, 12.82 per cent}.

Cobaltic trisBiguanidinium Carbonate.—The neutral carbonate was prepared by adding three molecular proportions of sodium bicarbonate to a cold concentrated solution of the complex base. The mixture was cooled in ice and the normal carbonate was salted out by the addition of a saturated solution of sodium carbonate. The crystals were washed and dried as before. The substance forms orange-yellow crystals, readily soluble in water, the solution being strongly alkaline to litmus. {Found: C, 18.50; Co, 12.12. $[\text{Co}(\text{BigH}^+)_3]_2(\text{CO}_3)_3$ requires C, 18.55; Co, 12.16 per cent}.

Cobaltic trisbiguanidinium sulphate was obtained in the form of sparingly soluble light red flakes when a solution of ammonium sulphate was added to that of the complex chloride. {Found: N, 37.09; S, 8.46;

Co, 10.40. $[\text{Co}(\text{BigH}^+)_3]_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ requires N, 37.11; S, 8.48; Co, 10.42 per cent}.

Cobaltic trisbiguanidinium selenate was obtained as moderately soluble light red flakes by treating the solid powdered base in a mortar with a cold solution of selenic acid. The crystals were filtered, washed with ice-water and then dried in air. {Found: Co, 9.21; Se, 18.51. $[\text{Co}(\text{BigH}^+)_3]_2(\text{SeO}_4)_3 \cdot 7\text{H}_2\text{O}$ requires Co, 9.26; Se, 18.65 per cent}.

Cobaltic trisbiguanidinium Chloro-selenate.—A cold saturated solution of the complex chloride was treated with a strong solution of potassium selenate. The mixture was strongly cooled and the precipitated crystals were washed and dried as usual. {Found: Cl, 6.0; Co, 10.0; Se, 13.25. $[\text{Co}(\text{BigH}^+)_3]_2 \text{SeO}_4 \text{Cl}$ requires Cl, 6.0; Co, 9.97; Se, 13.35 per cent}.

Cobaltic trisbiguanidinium dithionate was precipitated in the form of light red flakes when a cold saturated solution of the complex chloride was treated with a strong solution of sodium dithionate. The substance is moderately soluble in water. {Found: S, 15.84; Co, 9.85. $[\text{Co}(\text{BigH}^+)_3]_2(\text{S}_2\text{O}_8)_3$ requires S, 16.03; Co, 9.85 per cent}.

Cobaltic trisbiguanidinium Hydroxo-sulphite.—When a freshly prepared solution of sodium bisulphite was added, drop by drop, to an ice-cold concentrated solution of the base until the latter was partially neutralised, orange-yellow crystals of the hydroxo-sulphite separated from the solution. The crystals are moderately soluble in water giving an alkalinic solution. {Found: S, 6.72; Co, 12.0. $[\text{Co}(\text{BigH}^+)_3] \text{OH SO}_3$ requires S, 6.50; Co, 12.0 per cent}.

Cobaltic trisbiguanidinium Sulphite.—The normal sulphite was obtained as a reddish yellow crystalline precipitate by adding a freshly prepared concentrated solution of sodium sulphite to the cold concentrated solution of the complex chloride. The mixture was cooled in ice for some time and the crystals were washed and dried as usual. {Found: S, 8.74; Co, 10.68. $[\text{Co}(\text{BigH}^+)_3]_2(\text{SO}_3)_3 \cdot 7\text{H}_2\text{O}$ requires S, 8.85; Co, 10.88 per cent}.

Cobaltic trisbiguanidinium chloro-thiosulphate was precipitated in the form of sparingly soluble orange-coloured, silky crystals by the addition of a solution of sodium thiosulphate to that of the complex chloride. {Found: Cl, 6.24; S, 11.63; Co, 10.65. $[\text{Co}(\text{BigH}^+)_3]_2 \text{S}_2\text{O}_3 \text{Cl} \cdot 2\frac{1}{2}\text{H}_2\text{O}$ requires Cl, 6.43; S, 11.60; Co, 10.65 per cent}.

Cobaltic trisbiguanidinium Thiosulphate.—When a solution of the complex base was mixed with a solution of sodium thiosulphate and to the mixture a solution of ammonium acetate was added, light red silky crystals

of the normal thiosulphate were precipitated. {Found: S, 18.15; Co, 11.10. $[\text{Co}(\text{BigH}^+)_3]_2 (\text{S}_2\text{O}_3)_3$ requires S, 18.21; Co, 11.19 per cent}.

Cobaltic trisbiguanidinium chloro-chromate was obtained as a dark yellow crystalline precipitate when a solution of potassium chromate was added to that of the complex chloride. The crystals were washed with ice-water and dried over concentrated H_2SO_4 . {Found: Cl, 6.81; Co, 11.52; Cr, 10.48. $[\text{Co}(\text{BigH}^+)_3] \text{CrO}_4$ requires Cl, 6.95; Co, 11.55; Cr, 11.20 per cent}.

Cobaltic trisbiguanidinium Chromate.—When a solution of potassium dichromate was added to a solution of the complex base in the cold, sparingly soluble yellowish brown crystals of the normal chromate separated out. These were washed with cold water and dried in air. {Found: Co, 10.50; Cr, 13.72. $[\text{Co}(\text{BigH}^+)_3]_2 (\text{CrO}_4)_3 \cdot 3\text{H}_2\text{O}$ requires Co, 10.53; Cr, 13.92 per cent}.

Cobaltic trisbiguanidinium Perchromate.—When ammoniacal hydrogen peroxide (perhydrol) was added to a cold mixture of potassium chromate and the complex base in solution, dark yellowish brown crystals of the perchromate separated out after a short time. The crystals were washed with ice-cold water and dried over concentrated H_2SO_4 . {Found: Co, 9.57; Cr, 8.47; O, 11.4 (gasometrically), 12.4 (on correcting for chromate left in the solution after the evolution of oxygen). $[\text{Co}(\text{BigH}^+)_3] \text{CrO}_8 \cdot 4\text{H}_2\text{O}$ requires Co, 9.65; Cr, 8.47; O, 13.0 per cent}.

The perchromate decomposes, when acidified, giving rise to a blue solution; the blue colour disappears rapidly with the formation of green chromic salt.

Cobaltic trisbiguanidinium chloro-phosphate was obtained as a sparingly soluble, flesh-coloured crystalline precipitate by adding a solution of disodium hydrogen phosphate to a solution of the complex chloride. The crystals were washed first with cold water, then with alcohol and afterwards dried in air. {Found: Cl, 7.10; Co, 12.0; P, 6.35. $[\text{Co}(\text{BigH}^+)_3] \text{HPO}_4$ requires Cl, 7.23; Co, 12.02; P, 6.33 per cent}.

Cobaltic trisbiguanidinium phosphate was obtained as a light red powder by precipitating a solution of the complex base with a solution of disodium hydrogen phosphate. {Found: Co, 10.61; P, 8.24. $[\text{Co}(\text{BigH}^+)_3]_2 (\text{HPO}_4)_3 \cdot 6\text{H}_2\text{O}$ requires Co, 10.58; P, 8.34 per cent}.

Cobaltic trisbiguanidinium hydrosulphide was prepared by passing H_2S into a well-cooled slightly ammoniacal solution of the complex base. The precipitate formed was filtered and washed with ice-cold water. The substance decomposes on drying.

An analysis of the moist substance by oxidation with bromine gave: CoSO_4 , 0.1360 g.; BaSO_4 , 0.6057 g. Hence $\text{Co} : \text{S} = 1 : 3$. The molecular formula is, therefore, represented by $[\text{Co}(\text{BigH}^+)_3](\text{HS})_3$.

Cobaltic trisbiguanidinium hydro-polysulphide was obtained as a sparingly soluble crystalline precipitate by adding a solution of the yellow ammonium sulphide to that of the complex base. The crystals were washed at first with ice-cold water, then with alcohol and afterwards dried in vacuum over concentrated H_2SO_4 . {Found: S, 34.58; Co, 10.60. $[\text{Co}(\text{BigH}^+)_3](\text{HS}_2)_3$ requires S, 34.65; Co, 10.65 per cent}.

Cobaltic trisbiguanidinium iodate was prepared by treating the well-powdered complex base with a solution of iodic acid in a mortar. The reddish yellow crystals were filtered, washed and dried in air. The substance is fairly soluble in water. {Found: I, 42.93; Co, 6.61. $[\text{Co}(\text{BigH}^+)_3](\text{IO}_3)_3$ requires I, 43.09; Co, 6.65 per cent}.

Cobaltic trisbiguanidinium chloro-iodate was obtained as a yellow crystalline precipitate by treating a solution of the complex chloride with that of sodium iodate. The crystals were washed and dried as usual. {Found: Cl, 7.96; I, 27.34; Co, 8.41. $[\text{Co}(\text{BigH}^+)_3]_2(\text{IO}_3)_3 \cdot \text{Cl}_3 \cdot \text{H}_2\text{O}$ requires Cl, 7.74; I, 27.86; Co, 8.62 per cent}.

Cobaltic trisbiguanidinium periodate was obtained by precipitation from a solution of the complex chloride with sodium periodate. {Found: I (periodic), 20.42; Co, 9.50. $[\text{Co}(\text{BigH}^+)_3]\text{IO}_5 \cdot 3\text{H}_2\text{O}$ requires I, 20.48; Co, 9.51 per cent}.

Cobaltic trisbiguanidinium Oxalate.—Reddish yellow, fairly soluble crystals of the oxalate were obtained by treating the complex base with a cold concentrated solution of oxalic acid. {Found: Co, 12.0; C_2O_4 , 26.50. $[\text{Co}(\text{BigH}^+)_3]_2(\text{C}_2\text{O}_4)_3$ requires Co, 12.01; C_2O_4 , 26.88 per cent}.

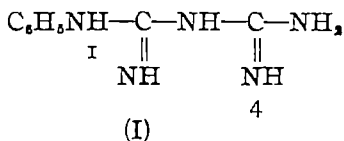
Cobaltic trisbiguanidinium camphorsulphonate was obtained as a flesh-coloured, moderately soluble crystalline precipitate by neutralising a cold concentrated solution of the complex base with a cold strong solution of camphorsulphonic acid. The crystals were washed first with ice-cold water and then with alcohol. They were dried over concentrated H_2SO_4 in vacuum. {Found: S, 9.12; Co, 5.71. $[\text{Co}(\text{BigH}^+)_3](\text{C}_{10}\text{H}_{16}\text{OSO}_3)_2$ requires S, 9.20; Co, 5.61 per cent}.

COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS. PART VII. COBALTIC *TRIS*-PHENYLBIGUANIDINES.

BY PRIYADARANJAN RÂY AND HARIHAR PRASAD BHATTACHARYA.

In continuation of the previous work on cobaltic *tris*biguandine and its derivatives, corresponding cobaltic complexes with a substituted biguanide—phenylbiguanide—have been prepared and their properties studied. Two different cobaltic *tris*phenylbiguanide hydrates have been obtained differing in their state of hydration and solubility in alcohol. Both the hydrates give the same cobaltic *tris*phenylbiguanidine on dehydration. A series of salts of the complex base—chloride, bromide, iodide, sulphate, nitrate, nitrite, carbonate, thiosulphate, thiocyanate, dithionate, and chromate—have been prepared and their properties described. They resemble the corresponding chromium compounds. A solution of the complex chloride has been found to give characteristic precipitates with many complex anions.

Phenylbiguanide (I) combines with trivalent cobalt like simple biguanide (*cf.* Part VI of this series) to form complex cobaltic trisphenylbiguanidine, its hydrate and salts. These resemble in composition and properties the corresponding chromium complexes (Rây and Ghosh, *J. Indian Chem. Soc.*, 1938, **15**, 350). As in the latter, the co-ordination with the trivalent cobalt atom occurs at (1) and co-valent binding at (4) for the same reasons as stated therein, and the constitution of the cobaltic trisphenylbiguanidines follows closely that of their chromium analogues.



Like chromium, trivalent cobalt also gives two modifications of cobaltic *trisphenylbiguanide* hydrate, one soluble and the other insoluble in alcohol. The anhydrous *trisphenylbiguanidine* as well as the di-hydrate have been found to be polymerised in alcoholic solution, due possibly to the formation of polynuclear complexes (*vide infra*).

EXPERIMENTAL.

Cobaltic trisPhenylbiguanide Trihydrate.—A solution of 5 g. of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in 25 c.c. of water was added gradually with shaking to 15 g. of phenylbiguanide hydrochloride, mixed with an excess of caustic soda solution (20 g.) in a 500 c.c. flask. The mixture was diluted to about

250 c.c. and a vigorous current of air was drawn through it, till the yellow precipitate of cobaltous phenylbiguanide, first formed, turned deep red in colour. Towards the latter part of the reaction, the flask was warmed on the water-bath when a pasty mass resulted. This was powdered in a mortar and washed with water till free from alkali. The precipitate was digested with absolute alcohol (75-100 c.c.) whereby a portion went into solution. With complete oxidation only a slight residue remains. The alcoholic solution was filtered and the filtrate, mixed with a little water, was kept overnight in the cold in a loosely corked conical flask. Red crystals, which separated, were filtered next morning. These were washed with alcohol and dried in vacuum over concentrated H_2SO_4 to a constant weight. The filtrate was preserved and used to extract the base in the next preparation, yield 2-3 g. A somewhat better yield was obtained by using caustic potash instead of caustic soda. {Found: N, 32.77, 32.98; Co, 9.25, 9.31. $[\text{Co}(\text{PhBig})_3] \cdot 3\text{H}_2\text{O}$ requires N, 32.76; Co, 9.20 per cent} where $\text{PhBigH} = 1$ molecule of phenylbiguanide.

The substance forms rose-red crystals, insoluble in water and alcohol. It absorbs carbon dioxide from atmosphere. Boiling water and alkali have no action upon the substance, but concentrated acids decompose it. It liberates ammonia from ammonium salt solutions on boiling and melts with decomposition at about 200° .

Cobaltic trisphenylbiguanide dihydrate was prepared by adding an excess of caustic soda solution to that of the complex chloride (*vide infra*). The resulting precipitate was washed with water and dried as described above. {Found: N, 33.2; Co, 9.54. $[\text{Co}(\text{PhBig})_3] \cdot 2\text{H}_2\text{O}$ requires N, 33.71; Co, 9.47 per cent}.

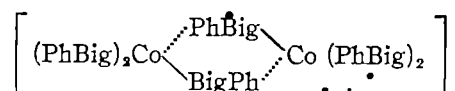
It forms pale rose crystals, insoluble in water but soluble in organic solvents, e.g., ethyl alcohol, methyl alcohol, acetone etc., giving a deep red solution. It, therefore, differs from the trihydrate in this respect. Like the latter it melts with decomposition.

Cobaltic trisPhenylbiguanidine.—The above described hydrates, when heated in an air-oven to 145° - 150° for nearly 24 hours, lost their water. The anhydrous compounds on exposure to air, however, readily absorb water; these are pale rose crystals, soluble in alcohol.

From the trihydrate :—Loss of water = 7.15; (calc. = 8.52 per cent). {Found: Co, 10.07. $[\text{Co}(\text{PhBig})_3]$ requires Co, 10.05 per cent}. The result for loss of water shows that the substance already lost a part of its water before drying in the oven.

From the dihydrate :—Loss of water = 5.97; (calc. = 5.78 per cent). {Found: Co, 9.97. $[\text{Co}(\text{PhBig})_3]$ requires Co, 10.05 per cent}.

Cobaltic trisphenylbiguanidine, prepared by dehydration from the tri- or dihydrate, gave, in alcoholic solution by the ebullioscopic method, a molecular weight of 1000-1200, which is nearly twice its normal molecular weight (590). The dihydrate in the same solvent gave a molecular weight of about 1700 against its normal value of 626. This indicates polymerisation, due possibly to the formation of polynuclear complexes. Thus for the anhydrous compound it may be represented by the formula,



The higher value in the case of dihydrate may be attributed to a similar polymerisation involving three cobalt atoms.

Cobaltic trisphenylbiguanidinium chloride was prepared by treating the solid base in a mortar with cold dilute HCl till the mixture became slightly acid. A clear solution was first obtained from which, after a few minutes, the crystals of the complex chloride separated all at once. The chloride tends to separate as an oil unless the solution is cooled. The crystals were washed with cold water, dissolved in alcohol and reprecipitated by the addition of pure acetone. The product was washed with acetone and dried over concentrated H_2SO_4 .

The substance forms needle-shaped orange crystals, soluble in water and alcohol, but insoluble in ether and acetone. {Found: N, 28.13; Cl, 14.40; Co, 7.98. $[\text{X}]\text{Cl}_3 \cdot 2.5 \text{H}_2\text{O}$ requires N, 28.32; Cl, 14.36; Co, 7.96 per cent} where $\text{X} = [\text{Co}(\text{PhBigH}^+)_3]$.

When heated to 110° for 15 hours the substance lost whole of its water and was converted into red anhydrous crystals.

Loss of weight = 6.04; Calc. water = 6.07 per cent.

Equivalent Conductivity at 35° .

v (dilution)	32	64	128	256	512	1024
$\lambda_{\infty} =$	103.3	106.1	112.1	118.8	124.9	133.1
$\lambda_{\infty} =$	141.3 (from Walden's formula).					

The valency of the complex ion from Ostwald-Bredig's rule = $\lambda_{1024} - \lambda_{32} = 133.1 - 103.3 = 2.98 \times 10$, i.e. 3.

Cryoscopic Measurement.

Subs. (g./100 c.c. calc. anhydrous).	Depression. Δ	Mol. wt. m .	van't Hoff's factor. $i = M/m$.	Degree of dissociation. $\alpha = i - 1/n - 1$.
0.5342	0.045°	213.7	3.26	0.75

Where M = mol. wt. calculated (anhydrous), n = number of ions.

Magnetic Susceptibility at 30°.

Subs.	$\chi_m \times 10^6$	$\chi_M \times 10^6$
[X] Cl ₃ ·2.5H ₂ O	-0.5501	-408.0 (dia)

The solution of the complex chloride gave coloured precipitates with a number of complex anions, e.g. ferrocyanide, ferricyanide, nitroprusside, cobalticyanide, platinichloride, mercuri-iodide, bismuthi-iodide, thiosulphato-cobalticyanide, disulphito-cobalticyanide, chromithiocyanate and sodium phosphomolybdate.

Cobaltic tris-phenylbiguanidinium bromide was obtained as a red crystalline precipitate by adding a concentrated solution of potassium bromide to that of the complex chloride in the cold. The crystals were washed with water and dried over concentrated H₂SO₄. {Found: Br, 28.27; Co, 6.95. [X]Br₃·H₂O requires Br, 28.28; Co, 6.96 per cent}.

It is moderately soluble in water and in alcohol.

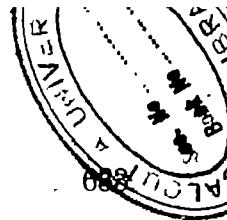
Cobaltic tris-phenylbiguanidinium iodide was prepared by double decomposition of the complex chloride and potassium iodide in the cold. The crystals, which separated, were washed with water whereby they changed into an oily mass. This was dissolved in alcohol and evaporated to dryness in vacuum over concentrated H₂SO₄, when a red glassy solid was obtained. {Found: I, 38.30; Co, 5.87. [X]I₃·H₂O requires I, 38.50; Co, 5.96 per cent}.

Cobaltic trisphenylbiguanidinium sulphate was prepared by digesting the complex base with a slight excess of dilute H₂SO₄ in a mortar and keeping the mixture overnight. The product was washed with water and dried in air. {Found: N, 25.62; S, 5.80; Co, 7.13. [X]₂(SO₄)₃·10H₂O requires N, 25.49; S, 5.82; Co, 7.16 per cent}.

The sulphate forms sparingly soluble red crystals.

Cobaltic trisphenylbiguanidinium nitrate was prepared by adding a concentrated solution of potassium nitrate to that of the complex chloride in the cold. The mixture, when cooled in ice, gave a crop of red crystals which were washed with cold water and dried over H₂SO₄. {Found: N, 32.80; Co, 7.53. [X](NO₃)₃· $\frac{1}{2}$ H₂O requires N, 32.10; Co, 7.52 per cent}.

COMPLEX COMPOUNDS OF BIGUANIDE



Cobaltic trisphenylbiguanidinium nitrite was precipitated by adding a strong solution of potassium nitrite to that of the complex chloride in the cold. The crystals were washed and dried as described above. {Found : N (nitritic), 5.65; Co, 8.0. $[X](NO_2)_3 \cdot \frac{1}{2}H_2O$ requires N, (nitritic), 5.70; Co, 8.0 per cent}.

Cobaltic trisphenylbiguanidinium carbonate was prepared from a concentrated solution of sodium carbonate and that of the complex chloride in the cold. The crystals were washed and dried as in the previous case. {Found : C, 43.80; Co, 8.38. $[X]_2(CO_3)_3 \cdot 2H_2O$ requires C, 43.96; Co, 8.47 per cent}.

The substance forms pale rose crystals, moderately soluble in water and gives a solution strongly alkaline to litmus.

Cobaltic trisphenylbiguanidinium thiosulphate separated as rose-coloured crystals when a concentrated solution of sodium thiosulphate was added slowly to a solution of the complex chloride. These were washed and dried as above. {Found : N, 25.05; S, 11.78. Co, 7.18. $[X]_2(S_2O_3)_3 \cdot 7H_2O$ requires N, 25.66; S, 11.73; Co, 7.21 per cent}.

Cobaltic trisphenylbiguanidinium thiocyanate was precipitated by adding a strong solution of potassium thiocyanate to a concentrated solution of the complex chloride in the cold. When washed with cold water the crystals readily turned into an oily mass. This was dissolved in alcohol and the solution was evaporated to dryness in vacuum over H_2SO_4 . A red glassy mass was obtained. {Found : Co, 7.28; S, 11.78. $[X](SCN)_3 \cdot 3H_2O$ requires S, 11.72; Co, 7.21 per cent}.

Cobaltic trisphenylbiguanidinium dithionate was obtained as pale rose crystals by mixing together strong solutions of the complex chloride and sodium dithionate. {Found : S, 11.24; Co, 6.99. $[X]_2(S_2O_8)_3 \cdot 2H_2O$ requires S, 11.32; Co, 6.97 per cent}.

Cobaltic trisphenylbiguanidinium chromate was precipitated in the form of pale rose crystals when a solution of sodium chromate was added to that of the complex chloride. {Found : Co, 7.55; Cr, 10.16. $[X]_2(CrO_4)_3 \cdot 2H_2O$ requires Co, 7.54; Cr, 9.97 per cent}.

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Received September 12, 1939.

COMPARISON OF THE CATAPHORETIC AND ELECTRO-OSMOTIC METHODS OF MEASURING ELECTROKINETIC POTENTIAL.

BY B. N. GHOSH AND P. C. ROY.

The electrokinetic potential of a number of suspensions has been measured by the cataphoretic and the electro-osmotic methods and the results obtained by the two methods have been found to agree between themselves.

It follows from theory that the electrokinetic potential of a given colloidal system should be the same whether it is measured by the cataphoretic method or by the electro-osmotic method. The velocity of electro-osmosis, U , is connected with the electrokinetic potential, e , of the capillary wall by the equation

$$U = C_1 \frac{qe_1ED}{\eta}$$

deduced by Smoluchowski, Perrin and Debye and Huckel, where $C_1 = 1/4\pi$, e , the electrokinetic potential of the capillary wall, E , the potential gradient, D , the viscosity of the dispersion medium and q , the cross section of the capillary. The cataphoretic velocity, V of a particle suspended in a liquid is given by the equation

$$V = C_2 \frac{e_2ED}{\eta}$$

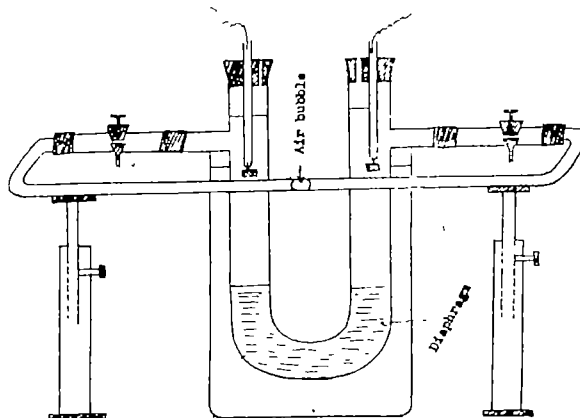
where $C_2 = 1/4\pi$ according to Smoluchowski, but according to Huckel it may be $1/4\pi$ or $1/6\pi$ depending on whether the particle is cylindrical or spherical in shape. The other terms have the same significance as in the case of electro-osmosis. For a given system U/V should, therefore, be unity according to Smoluchowski's equation and 1.5 according to Hückel's equations, provided the particles are spherical. This point has been subjected to experimental test by van der Grinten (*J. chim. phys.*, 1926, **23**, 209), by Henry (*Proc. Roy. Soc.*, 1931, **A**, **133**, 106) and by Bull and Gortner (*J. Phys. Chem.*, 1932, **36**, 111). Van der Grinten using a microcataphoretic cell of glass and particles formed of the same glass of which the cell is made, measured the cataphoretic velocity, V , of the particles and also the velocity of electro-osmosis of water, U , in the cell due to the electric charge of the cell wall. He obtained for U/V the value

1'5. His results have, however, been adversely criticised on the ground that the glass particles prepared by grinding have not the same surface as that of the cell. To overcome this difficulty Bull and Gortner suspended the glass particles in a dilute protein solution and measured U and V in a microcataphoretic cell of glass. They assumed that the surfaces of the cell and those of the particles were uniformly coated with a film of protein. Under these conditions they found U/V to be approximately equal to 1. Henry (*loc. cit.*) in his experiments, used spherical wax particles and coated the surface of the cataphoretic cell with the same wax. He also found U/V to be nearly equal to unity. In all these experiments the assumption is made that as a result of the wax or protein coating the surface structure of the particles has become identical with that of the cataphoretic cell. It is doubtful how far such an assumption is correct. This doubtful factor can, however, be eliminated if a porous diaphragm is prepared of the same particles, the cataphoretic velocity of which is being measured and U , the electro-osmotic velocity is determined by making use of this diaphragm. This procedure has been adopted in our experiments. The values of U and V were determined for particles of kaolin, kaolin coated with gelatin, silica, silica coated with fibrin, arsenious sulphide, etc. in contact with solutions of various electrolytes. The results obtained so far are recorded in this paper.

EXPERIMENTAL.

The kaolin or silica particles used in these experiments were prepared by allowing their aqueous suspensions about 6 inches in depth to stand in glass bottles and collecting the particles which settle to the bottom between two and four hours. The particles were coated with proteins, when desired, by shaking them with dilute solutions of proteins, removing the

FIG. 1.



supernatant solution after the sedimentation of the particles and shaking them again with fresh solutions of proteins and so on until their surface was saturated. The arsenious sulphide particles used were obtained by treating an arsenious sulphide solution with electrolytes of such concentration that the coagulation of the solution occurred in about three hours.

The arrangements used for the electro-osmotic experiments were similar to that used by Mukherjee and co-workers (*Nature*, 1922, 110, 732) and also by Ghosh (*J. Chem. Soc.*, 1929, 2693). It consisted of a U-shaped tube, the side tubes of which were connected with a narrow tube bent four times at right angles, through two three-way glass stop-cocks (Fig. 1). The lower part of the U-tube was packed with the material which formed the diaphragm. The upper part of the U-tube and the bent tube were filled with solutions of electrolytes with which the particles forming the diaphragm were previously kept in contact for a time sufficiently long for the attainment of adsorption equilibrium. The bent tube connected with the U-tube contained an air-bubble in the middle. From a measurement of the movement of this air-bubble, when the electric field is applied, the volume of the liquid flowing through the diaphragm per second can be calculated, provided the diameter of the bore of the bent tube is known. In the equation

$$U = C_1 \frac{q e_1 E D}{\eta},$$

q represents the cross section of the capillary tube. If instead of a single capillary tube a porous diaphragm is used, we may regard it as a bundle of capillaries of effective cross section q . Now $E = i_1 R$, where i_1 is the current and R the resistance per unit length of the capillaries. Hence $R = \frac{1}{qk}$ where k is the specific conductivity of the liquid filling the pores of the diaphragm. Substituting this value of E in the above equation we get

$$U = C_1 \frac{i_1 e_1 D}{\eta k} \quad \text{or} \quad U/i_1 = C_1 \frac{e_1 D}{\eta k} \quad \dots (1)$$

The cataphoretic velocities of the particles were determined in a micro-cataphoretic cell of the flat rectangular type used by Freundlich and Abramson (*Z. physikal. Chem.*, 1927, 128, 25) and by Chopra and Chaudhury (*Indian J. Med. Res.*, 1932, 19, 1115) with this modification that it was provided with two detachable side-tubes, one on each side. The joints were carefully ground so as to be leak-proof. Each side-tube carried an electrode vessel and a three-way stop-cock. The cell was cleaned thoroughly before use and then filled with the desired suspension, care being taken to remove all air-bubbles from the apparatus. Reversible electrodes

were placed in the electrode vessels and just before taking actual reading they were connected with the 220 volt lighting circuit through a sensitive milliammeter and an adjustable resistance. The milliammeter indicated the current which flowed through the system. The velocity of the particles were measured at two depths $d/6$ and $d/2$ respectively inside the cell, where d represents the depth of the cell. The value of d for the cell, used by us was 0.09 cm. The actual velocity of the particles was calculated from the formula,

$$V = (\frac{2}{3}V_1 + \frac{1}{3}V_2),$$

where V_1 and V_2 are the observed velocities at depths $d/6$ and $d/2$ respectively inside the cell as already stated. Now V is related to the electrokinetic potential by the equation,

$$V = \frac{C_2 e_2 D E}{\eta} = \frac{C_2 e_2 D i_2}{\eta A k} \quad \dots (2)$$

Since $E = i_1 R$, where i_2 is the current and R , the resistance per unit length of the cataphoretic cell and since $R = i_2 / A k$ where A is the cross sectional area of the cataphoretic cell and k , the specific conductivity of the suspension filling it.

Hence from equation (2) we get

$$\frac{V A}{i_2} = \frac{C_2 e_2 D}{\eta k} \quad \dots (3)$$

From equations (1) and (3) it follows that for the same suspension for which D , η and k have identical values

$$\frac{(V A)}{i_2} / (U / i_1) = \frac{C_2}{C_1} \cdot \frac{e_2}{e_1} \quad \dots (4)$$

TABLE I.

The cross sectional area 'A' of the cataphoretic cell = 0.09 sq.cm.

Material used.	Specific conductivity.	Current in milliamperes i_2	$\frac{V.A}{i_2}$	Current in milliamperes i_1	U/i_1	$\frac{e_2}{e_1} \times \frac{C_2}{C_1}$
Kaolin treated with HCl of diff. conc	3.9×10^{-3}	3.25	0.0369	14	0.379	0.97
	1.05×10^{-3}	1.05	0.227	6	0.207	1.09
Gelatin coated kaolin and HCl of diff. conc	1.03×10^{-1}	11.5	0.0305	10	0.028	1.09
	3.22×10^{-3}	3.00	0.0321	14	0.029	1.11
Silica and HCl of diff. conc.	7.74×10^{-3}	4.5	0.0182	10	0.0226	0.81
	1.19×10^{-3}	1.1	0.187	11	0.182	1.03
Fibrin coated silica and HCl of diff. conc	1.6×10^{-3}	1.5	0.170	12	0.142	1.19
	3.2×10^{-4}	0.72	0.318	7	0.282	1.13
Arsenious sulphide						
treated with BaCl_2 soln.	2.5×10^{-4}	0.7	0.401	3	0.407	0.99
CaCl_2 soln.	3.4×10^{-4}	0.6	0.535	7.3	0.492	1.09
Mean 1.05						

It appears from the data recorded in Table I that the value of $C_2 e_2 / C_1 e_1$ is unity within the limits of experimental error. If it be assumed that $e_2 = e_1$ then it follows from our results that within the limits of experimental error $c_2 = c_1 = 1/4\pi$ i.e. C_2 as required by Smoluchowski's equation is independent of the shape of the particles. The assumption that $e_2 = e_1$ is justifiable since the same suspensions were used in the cataphoretic and electro-osmotic measurements. This result is useful in view of the fact that it demonstrates the reliability, provided proper precautions are taken of electro-osmotic method of measuring the electrokinetic potential of particles which settle quickly from their suspensions, thereby rendering the cataphoretic method of measuring electrokinetic potential rather difficult in their case. It may be mentioned here that in evaluating the electrokinetic potential from the experimental data, it has been assumed by us that the specific conductivity 'k' of the electrolyte solution within the pores of the diaphragm is identical with its specific conductivity in bulk, in other words, the effect due to surface conduction inside the pores is negligible. This assumption seems justifiable in view of the fact that the solutions used by us were fairly strongly conducting and the diameters of the pores were large, owing to the comparatively loose packing of the material forming the diaphragm.

SYNTHETICAL EXPERIMENTS IN THE PYRONE SERIES. ATTEMPTED OXIDATION OF CHROMANONES WITH SELENIUM DIOXIDE. PART I.

BY DUKKHAHARAN CHAKRAVARTI AND JYOTIRMOY DUTTA.

The chromanones are not oxidised with selenium dioxide to the chromones, though the flavanones and chalcones are oxidised with selenium dioxide to the flavones.

The observation of Venkataraman and co-workers (Mahal, Rai and Venkataraman, *J. Chem. Soc.*, 1935, 866; Mahal and Venkataraman, *ibid.*, 1936, 569) that certain flavanones or even some chalcones are oxidised to flavones by selenium dioxide led the authors to attempt the oxidation of chromanones with selenium dioxide for the unambiguous synthesis of substituted chromones which cannot be synthesised by the methods known at present.

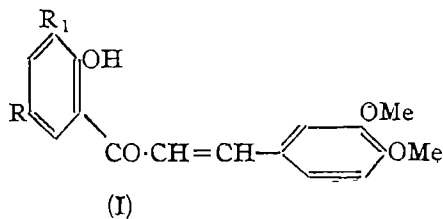
The chromanones have been obtained by the cyclisation of the phenoxypropionic acids using phosphorus pentoxide in benzene solution. The phenoxypropionic acids have been prepared by following the method of Perkin, Rây and Robinson (*J. Chem. Soc.*, 1926, 941; Perkin, Pollard and Robinson, *ibid.*, 1937, 49) by reacting *o*- and *p*-chlorophenol, *o*- and *p*-nitrophenol, *o*- and *p*-cresols, α - and β -naphthol with β -chloropropionic acid in alkaline medium. It should be noted that when attempts are made for cyclisation by 85% sulphuric acid, the yields are very poor and in some cases the phenols are sulphonated. The chromanones have been characterised by condensing them with veratraldehyde in presence of dry hydrochloric acid in acetic acid solution forming 3-veratrylidene derivatives. The α -naphthachromanone, prepared by this method, has been found to be identical with the compound obtained by Pfeiffer and Grimmer (*Ber.*, 1917, 50, 911) from 2-acetyl-1-naphthol.

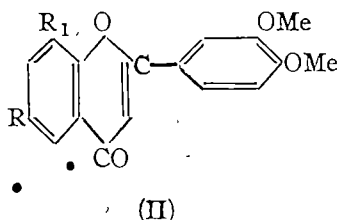
The oxidation of the chromanones is, however, attended with difficulties. In view of the observation of Chakravarti and co-workers (*J. Indian Chem. Soc.*, 1931, 8, 129, 407, 619; 1932, 9, 25, 31, 389; 1936, 31, 619, 649) that the halogen atom and the nitro group favour γ -pyrone formation in Simons' reaction, it was thought that the halogenated

chromanones, *e.g.*, 6-chlorochromanone, would give the corresponding chromone more easily. 6-Chlorochromanone has been oxidised with selenium dioxide, but with negative results. All attempts to synthesise the chromones by the oxidation of the chromanones with selenium dioxide under varying conditions of temperature and in different solvents, *e.g.*, xylene, rectified spirit, amyl alcohol, have been unsuccessful. In all cases metallic selenium is precipitated and when the product is treated with dilute alkali a coloured solution is obtained, which on acidification gives an amorphous yellow substance, but the latter does not freely dissolve in alkali and resists all attempts at crystallisation. The identity of these yellow substances could not be established but qualitative tests prove the presence of a high percentage of selenium in these substances. When the reactions are done in amyl alcoholic solution crystalline substances containing selenium separate. The presence of an *ortho*-diketone, one of the possible products of oxidation, could not be detected.

It was thought that the halogen atom instead of exerting a favourable influence might conceivably interfere, and hence nitrochromanones have been oxidised but with no better results. In order to eliminate the unfavourable influence of the halogen atom or the nitro group, if any, the methylchromanones have been oxidised but with the same result.

These unsuccessful attempts for the oxidation of the different chromanones led the authors to doubt the general applicability of Venkataraman's method for the oxidation of flavanones and chalkones, and hence chalkones containing halogen atom and nitro group in the benzene nucleus have been prepared and oxidised with selenium dioxide; but in conformity with Venkataraman's observations the oxidation is effected very smoothly with the formation of flavones in highly satisfactory yield. The chalkones are evidently transformed into flavanones which undergo oxidation. Thus the following chalkones (I) have been oxidised to the corresponding flavones (II): 3',4'-dimethoxy-2-hydroxy-5-chlorochalkone (I, R=Cl; R₁=H); 3',4'-dimethoxy-2-hydroxy-3-chlorochalkone (I, R=H, R₁=Cl); 3',4'-dimethoxy-2-hydroxy-3-nitro-6-methylchalkone (I, R=Me; R₁=NO₂).





In view of the fact that the chromanones are not oxidised, while the chalcones, irrespective of any substituent in the benzene nucleus, undergo smooth oxidation with selenium dioxide without the formation of selenium complexes, it is evident that the phenyl group in the pyrone nucleus is responsible for this smooth oxidation.

E X P E R I M E N T A L.

General Method for the Preparation of the Phenoxypropionic Acids.—The phenol (1 mol.) was dissolved in caustic potash solution (30 %) and heated on the water-bath. The amount of the caustic potash used was about twice the amount required to neutralise the phenol. The solution of the neutralised β -chloropropionic acid (1 mol.) was then added in small quantities at a time to the heated solution during 1 hour and the reaction mixture further heated for 2-4 hours. The cold solution was acidified with hydrochloric acid and extracted with ether and the ethereal extract washed with sodium bicarbonate solution and the latter acidified with hydrochloric acid. The precipitate was collected, again dissolved in sodium bicarbonate solution and the precipitate obtained on acidification was crystallised from a solvent.

General Method for the Preparation of the Chromanones.—The phenoxypropionic acid (1 part) was dissolved in benzene (20 parts) and the solution refluxed with phosphorus pentoxide (3 parts) for 10 hours. The supernatant benzene layer was then decanted off and the pasty residue was decomposed with powdered ice and extracted with hot benzene. The benzene extract was washed with sodium bicarbonate solution and then with water and dried and on removal of benzene the residue was either crystallised from a suitable solvent or distilled in *vacuo*. The phenoxypropionic acids and the chromanones prepared are described in Table I.

3' : 4'-Dimethoxy-2-hydroxy-5-chlorochalkone.—A solution of 2-oxy-5-chloroacetophenone (4 g.) in alcohol (50 c.c.) was treated with caustic potash (50%, 15 c.c.) and the red solution was refluxed on the water-bath for 1 hour. The solution was cooled, acidified and the precipitate crystallised from glacial acetic acid as reddish orange needles, m.p. 174°, yield 3 g. It gives a brown colour with ferric chloride. (Found : Cl, 11.35. $C_{17}H_{15}O_4Cl$ requires Cl, 11.15 per cent).

3' : 4'-Dimethoxy-6-chloroflavone was obtained by the oxidation of the above compound (2 g.) in amyl alcohol (30 c.c.) by selenium dioxide (2 g.) by refluxing at 150-60° for 12 hours. The solution was filtered hot from the precipitated metallic selenium when on cooling crystals appeared. It was recrystallised from glacial acetic acid as yellow needles, m.p. 194°, yield 1.5 g. It gives no colouration with ferric chloride. (Found : Cl, 11.40. $C_{17}H_{13}O_4Cl$ requires Cl, 11.21 per cent).

3' : 4'-Dimethoxy-2-hydroxy-3-chlorochalkone was obtained from 2-hydroxy-3-chloroacetophenone (4 g.) and veratraldehyde (4 g.) as in the previous case. It crystallised from alcohol as orange plates, m.p. 163-64°, yield 4 g. (Found : Cl, 11.45. $C_{17}H_{13}O_4Cl$ requires Cl, 11.15 per cent).

3' : 4'-Dimethoxy-8-chloroflavone was obtained from 3' : 4'-dimethoxy-2-hydroxy-3-chlorochalkone (2 g.) by oxidation with selenium dioxide (2 g.) in amyl alcohol (20 c.c.). On removal of amyl alcohol from the filtered solution by steam distillation the residue was crystallised from benzene as chocolate needles, m.p. 110° (decomp.), yield 1 g. It gives an ash-coloured precipitate with ferric chloride. (Found : Cl, 11.48. $C_{17}H_{13}O_4Cl$ requires Cl, 11.21 per cent).

3' : 4'-Dimethoxy-2-hydroxy-3-nitro-5-methylchalkone, prepared from 2-hydroxy-3-nitro-5-methylacetophenone (4 g.) and veratraldehyde (4 g.), crystallised from dilute acetic acid as chocolate needles with a green reflex, m.p. 175°, yield 4 g. It gives a blood-red colouration with ferric chloride. (Found : N, 4.13. $C_{18}H_{17}O_6N$ requires N, 4.08 per cent).

3' : 4'-Dimethoxy-6-methyl-8-nitroflavone was obtained from the above compound (2 g.) by oxidation with selenium dioxide (2 g.) in amyl alcohol (40 c.c.). The hot solution deposited crystals on cooling which were recrystallised from amyl alcohol as brown needles, m.p. 244-45° (decomp.), yield 1 g. (Found : N, 4.23. $C_{18}H_{15}O_6N$ requires N, 4.11 per cent).

TABLE I

Name.	Formula.	Prepared from β -chloro-propionic acid and	M. p.	Analysis Found.	Calc.	Remarks.
β -(<i>p</i> -Chloro)-phenoxy-propionic acid (I)	$C_8H_9O_3Cl$	<i>p</i> -Chlorophenol	138-39°	Cl, 18.05%	17.71	Shining plates from alcohol
6-Chlorochromanone	$C_9H_7O_3Cl$	(I) & P_2O_5	106°	20.0	19.5	Light yellow needles from alcohol
3-Veratrylidene derivative	$C_{18}H_{15}O_4Cl$	—	151-52°	10.96	10.74	Orange plates from alcohol
β -(<i>o</i> -Chloro)-phenoxy-propionic acid (II)	$C_9H_9O_3Cl$	<i>o</i> -Chlorophenol	108-9°	17.6	17.71	Colourless needles
8-Chlorochromanone	$C_9H_7O_3Cl$	(II) & P_2O_5	65°	19.8	19.5	Light yellow needles
3-Veratrylidene derivative	$C_{18}H_{15}O_4Cl$	—	110-11°	10.85	10.74	Yellow needles
β -(<i>p</i> -Nitro)-phenoxypropionic acid (III)	$C_9H_9O_6N$	<i>p</i> -Nitrophenol	118-19°	N, 6.98	6.64	Grey needles
6-Nitrochromanone	$C_9H_7O_4N$	(III) & P_2O_5	176-77°	N, 7.23	7.25	Dirty white needles
3-Veratrylidene derivative	$C_{18}H_{15}O_4N$	—	190-91°	N, 4.18	4.11	Yellow needles
β -(<i>o</i> -Nitro)-phenoxypropionic acid (IV)	$C_9H_9O_5N$	<i>o</i> -Nitrophenol	121-22°	N, 7.0	6.64	Grey needles from water
8-Nitrochromanone	$C_9H_7O_5N$	(IV) & P_2O_5	126-27°	N, 7.28	7.25	Light yellow needles
3-Veratrylidene derivative	$C_{18}H_{15}O_4N$	—	179-80°	N, 4.43	4.11	Yellow needles from acetic acid
* β -(2-) Naphthoxypropionic acid (V)	$C_{15}H_{17}O_3$	β -Naphthol	144-45°	C, 72.40 H, 5.89	72.22 5.55	Colourless needles
β -Naphthachromanone	$C_{13}H_{10}O_3$	(V) & P_2O_5	b. p. 185-87/9mm.	C, 78.91 H, 5.64	78.79 5.56	Yellow oil
*Semicarbazone	$C_{14}H_{13}O_3N_3$	—	m. p. 227° (decomp.)	N, 16.63	16.47	Light yellow plates from alcohol

TABLE I (contd.).

Name.	Formula.	Prepared from β -chloro-propionic acid and	M.p.	Analysis Found : Theo .	Remarks
* β -(1-Naphthoxy)propionic acid (VI)	$C_{13}H_{13}O_3$	α -Naphthol	147-48°	C, 72.40 H, 5.62	Identical with the compound described by Pfeiffer and Grimmer (<i>loc cit</i>)
α -Naphthachromanone	$C_{13}H_{10}O_2$	(VI) & P_2O_5	104°	—	Light yellow needles, identical with the compound described by Pfeiffer and Grimmer (<i>loc cit</i>)
*3-Veratrylidene derivative	$C_{22}H_{18}O_4$	—	169-70°	C, 76.21 H, 5.24	Greenish yellow needles from acetic acid
* β -(α -Methyl)-phenoxy-propionic acid (VII)	$C_{10}H_{13}O_3$	α -Cresol	94.5°	C, 67.14 H, 7.05	Greyish needles
8-Methylchromanone	$C_{10}H_{10}O_2$	(VII) and P_2O_5 b.p. 125-30°/9mm.		C, 74.14 H, 6.30	•Light yellow liquid
Semicarbazone	$C_{11}H_{13}O_2N_3$		m p. 230-31° (decomp)	N, 19.32	Yellow needles
β -(p -Methyl)-phenoxy-propionic acid (VIII)		p -Cresol	146°	} Identical with the compounds described by Kroll-pfeiffer and Schultze (<i>Her</i> , 1924, 87, 206)	
6-Methylchromanone	—	(VIII) and P_2O_5 b p 118-26°/6mm			
*3-Veratrylidene derivative of 6-methylchromanone	$C_{19}H_{18}O_4$		m.p 131-32°	C, 73.13 H, 5.72	Orange plates from alcohol

The analyses of compounds marked with * are micro-analyses, done by Mr. N. Ghosh, M.Sc., to whom our best thanks are due.

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Received September 31, 1939

MUTUAL COAGULATION OF COLLOIDAL SOLUTIONS. INTERACTION OF COPPER FERROCYANIDE WITH FERRIC HYDROXIDE, THORIUM HYDROXIDE AND CERIC HYDROXIDE.

BY P. M. BARVE, V. C. VORA* AND B. N. DESAI.

Mutual coagulation of the pairs copper ferrocyanide and ferric hydroxide, copper ferrocyanide and thorium hydroxide and copper ferrocyanide and ceric hydroxide has been studied. It is observed that the width of the zone of mutual coagulation is minimum when the charge on the colloidal particles of the reacting sols is maximum, the value of the minimum width also depending upon the hydration of the particles. The width of the zone of mutual coagulation seems to be mainly controlled by the charge on the colloidal particles, the fact whether the peptising electrolytes and other impurities present in the sols chemically react or not on mixing them being unimportant.

In previous papers (Barve, Paranjpe and Desai, *J. Bombay Univ.*, 1937, 6, ii, 50; 1939, 8, ii, 134), it has been shown that in the case of mutual coagulation of Prussian blue and (a) ferric hydroxide, (b) thorium hydroxide and (c) ceric hydroxide with the progress of dialysis, the width of the zone of mutual coagulation first decreases and then increases, the charge on the particles of the sols in all the cases first increasing and then decreasing at the same time. The sols tried so far were all acidic and the peptising electrolytes in them were such that on mixing the sols no chemical interaction took place. In the present case copper ferrocyanide has been substituted for Prussian blue as the former is basic, while the other sols have been kept the same, in order to study the effect of chemical interaction between the stabilising ions and to see if the same relation between the width of the zone of mutual coagulation and the charge on the colloidal particles of the sols as observed before holds good.

EXPERIMENTAL.

Copper ferrocyanide sol was prepared in the same manner as done by Chaudhury (*J. Indian Chem. Soc.*, 1933, 10, 431) and ferric hydroxide, thorium hydroxide and ceric hydroxide as done by Desai and co-workers (*loc. cit.*). Details of dialysis and charge measurements were the same as given in earlier papers.

* The experimental work has been carried out entirely by Mr. V. C. Vora under the direction of the other two authors.

The zone of mutual coagulation was first determined roughly by finding out in each case the amounts of the two reacting sols at which turbidity first appears and then disappears, the total volume of the mixture being kept 10 c.c. The zone of complete coagulation was afterwards accurately determined by taking a number of mixtures in the boundary region of the zone previously roughly determined, by varying the amount of the colloids by 0.05 c.c. The mixtures were then centrifuged for 3 minutes at about 2500 r.p.m. and examined for complete coagulation. When the presence of a small amount of colloid was not readily determined in the supernatant liquid by visual observation, the latter was pipetted off and treated with an excess of suitable electrolyte solutions. The absence of any turbidity on standing was taken as an indication that no colloid was present in the supernatant liquid.

The results of charge measurements are given in Table I and those of the determination of the zone of mutual coagulation in Tables II-IV.

TABLE I.

Ca taphoretic speed $\times 10^5$.

Dialysed for	Copper ferrocyanide.	Ferric hydroxide.	Thorium hydroxide	Ceric hydroxide.
0 days	16.9	26.3	28.9	14.3
3	—	36.1	34.0	—
4	22.6	—	—	18.1
7	—	47.6	38.1	—
8	29.4	—	—	26.4
11	—	63.4	41.3	—
12	38.5	—	—	33.6
14	—	78.8	42.6	—
15	46.2	—	—	42.9
18	—	41.9	32.4	—
19	34.2	—	—	33.2
22	—	29.3	27.5	—
23	23.7	—	—	21.7

TABLE II.

Mutual coagulation of copper ferrocyanide by ferric hydroxide.

Numbers in different columns refer to c.c. of ferric hydroxide in 10 c.c. of the mixture of copper ferrocyanide and ferric hydroxide. A = Appearance of turbidity. Z = Zone of coagulation. D = Disappearance of turbidity.

Days of dialysis of copper ferrocyanide.

	0			3			7			11			14			18			22		
	A	Z.	D.	A.	Z.	D.	A.	Z.	D.	A.	Z.	D.	A.	Z.	D.	A.	Z.	D.	A.	Z.	D.
0	0.6	8.4	9.0	0.7	7.0	7.7	1.0	5.8	6.8	1.2	3.2	4.4	1.7	2.1	3.8	1.4	2.9	4.3	0.9	3.5	4.4
3	0.8	8.2	9.0	0.9	<u>6.5</u>	7.4	1.1	5.2	6.3	1.3	2.9	4.2	1.9	1.7	3.6	1.3	3.4	4.7	0.8	4.1	4.9
7	0.9	8.4	9.3	1.0	6.1	7.1	1.3	<u>4.7</u>	6.0	1.1	2.6	3.7	2.1	1.6	3.7	1.1	3.9	5.0	0.8	4.8	5.6
11	0.7	8.5	9.2	0.6	6.8	7.4	1.2	4.9	6.1	0.9	2.0	2.9	2.4	1.5	3.9	0.9	4.4	5.3	0.6	5.4	6.0
14	1.0	8.6	9.6	0.8	6.9	7.7	1.0	5.3	6.3	1.0	2.9	3.9	2.6	<u>1.1</u>	3.0	0.7	4.5	5.2	0.5	5.9	6.4
18	1.1	8.7	9.8	0.9	7.1	8.0	0.8	5.7	6.5	1.4	3.1	4.5	1.8	3.1	4.9	0.8	<u>4.8</u>	5.6	0.7	6.5	7.2
22	0.9	8.9	9.8	0.7	7.4	8.1	0.9	6.0	6.9	1.3	3.8	5.1	1.3	4.7	5.0	0.9	5.3	6.2	0.9	<u>6.9</u>	7.8

Days of dialysis of ferric hydroxide

TABLE IV.

Mutual coagulation of copper ferrocyanide by ceric hydroxide.

Numbers in different columns refer to c.c. of ceric hydroxide in 10 c.c. of the mixture of copper ferrocyanide and ceric hydroxide.

Days of dialysis of ceric hydroxide

	0			4			8			12			15			19			23		
	A	Z.	D	A	Z.	D.	A	Z	D	A	Z	D	A	Z	D	A	Z	D	A	Z.	D
0	1.4	8.0	9.4	1.2	7.5	8.7	1.1	6.5	7.6	1.6	5.8	7.4	1.4	5.1	6.5	1.1	6.1	7.2	0.9	7.4	8.3
4	1.3	8.2	9.5	1.0	<u>7.1</u>	8.1	1.6	6.6	8.2	1.4	6.0	7.4	1.2	5.6	6.8	1.0	<u>6.3</u>	7.3	0.8	7.6	8.4
8	1.6	8.0	9.6	1.3	7.4	8.7	2.0	<u>6.7</u>	8.7	1.5	6.2	7.7	1.8	5.0	<u>6.8</u>	1.0	6.7	7.5	1.0	7.8	8.8
12	1.4	8.3	9.7	1.2	7.3	8.5	1.8	6.4	8.2	1.3	<u>6.5</u>	7.8	2.2	4.9	7.1	0.9	7.1	8.0	0.7	8.0	8.7
15	1.3	8.4	9.7	1.0	7.8	8.8	1.4	6.1	7.5	1.2	5.9	7.1	2.8	<u>4.2</u>	7.0	1.3	6.4	7.7	0.9	8.1	9.0
19	1.2	8.6	9.8	1.2	8.0	9.2	1.0	6.8	7.8	1.1	5.6	6.7	2.0	4.8	6.8	1.4	<u>6.8</u>	8.2	1.0	8.0	9.0
23	1.2	8.7	9.8	1.1	8.3	9.4	1.1	6.9	8.0	1.0	5.4	6.4	1.3	5.8	7.1	1.0	7.0	8.0	0.9	8.2	9.1

Days of dialysis of copper ferrocyanide

DISCUSSION.

From Table I it will appear that in all the cases with the progress of dialysis, the cataphoretic speed, *i.e.* the charge on the colloidal particles, first increases, reaches a maximum and then decreases. The initial increase in cataphoretic speed is due to some of the oppositely charged ions which are bound with the primarily adsorbed, *i.e.* preferentially adsorbed similarly charged ions, as a result of electrical attraction, becoming free as their concentration in the sol diminishes during dialysis; the final decrease in cataphoretic speed is due to desorption of the preferentially adsorbed similarly charged ions from the surface of the particles, *i.e.* from the inner sheet of the double layer as their concentration in the intermicellary liquid becomes very low in the latter stages of dialysis (Desai, Barve and Paranjpe, *Proc. Roy. Soc. Edm.*, 1939, 89, 22).

It will appear from Tables II, III and IV that when to one of the sols dialysed for a certain period, another sol dialysed for different periods is added, the changes in the range (value of Z) within which mutual coagulation takes place are not regular. This is to be expected as sols dialysed for different periods contain different amounts of impurities and they will change the charge on the particles differently on mixing.

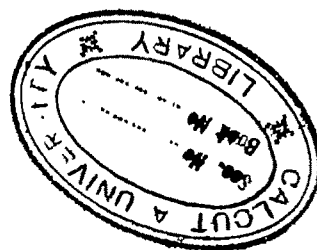
If we consider the range within which mutual coagulation occurs for both the sols dialysed for the same periods, it appears that with the progress of dialysis, it (value of Z underlined in Tables II, III and IV) first decreases, reaches a minimum value and then increases. For the pairs copper ferrocyanide and ferric hydroxide, copper ferrocyanide and thorium hydroxide and copper ferrocyanide and ceric hydroxide, the smallest value of the width of the zone of mutual coagulation has been 1.4, 1.9 and 4.2 units respectively occurring for sols dialysed for 14 to 15 days. From Table I it is seen that the maximum value of charge has also occurred for sols dialysed for about the same period. It is thus clear that even when the two reacting sols contain peptising electrolytes and other impurities, which may chemically react on mixing them, the width of the zone of mutual coagulation is mainly controlled by charge on the particles of the sols. When the charge on the particles of one of the sols, which is in excess, is highest, the amount of the other oppositely charged colloid required to be added to coagulate it, will have to be sufficiently large so as to lower down the charge to such a value that aggregation can take place, producing turbidity; similarly the disappearance of turbidity or its appearance from the other end (*i.e.* when the second colloid is in excess and its particles have the highest charge) will

occur only when large amount of the coagulating colloid from that end is added. Thus the zone of precipitation will be the narrowest, *i.e.*, the value of Z will be the smallest when the particles of both the sols have greatest charge.

As mentioned before, the smallest value of the width of the zone of mutual coagulation has been 1.4, 1.9 and 4.2 in the case of the pairs copper ferrocyanide and (a) ferric hydroxide, (b) thorium hydroxide and (c) ceric hydroxide respectively. This difference in the values is probably due to a difference in hydration of the colloid particles of the three sols, the ceric hydroxide particles being decidedly more hydrated than those of ferric hydroxide or thorium hydroxide. This effect of hydration of the colloid particles has also been noticed in the case of other sols studied in this laboratory (Barve, Vora and Desai, *loc. cit.*).

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Received August 29, 1939.



CHROMIUM CHROMATE.

BY PRODOSH CHANDRA ROY CHAUDHURY.

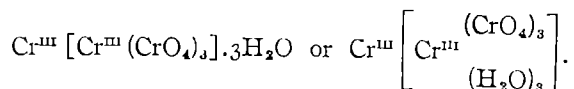
Chromium chromate has been prepared from chromic chloride solution and silver chromate by double decomposition. The substance forms a brown glassy mass in the dry state, which is soluble in water. A study of the physical properties of its aqueous solution, such as the measurement of equivalent conductivity, freezing point, p_H value, magnetic susceptibility and absorption spectra, as well as of its chemical properties, shows that it is not a simple chromic chromate, $\text{Cr}_2(\text{CrO}_4)_3 \cdot 3\text{H}_2\text{O}$, but contains a complex chromato-chromiate anion and should be represented as $\text{Cr} \left[\text{Cr} \begin{smallmatrix} (\text{CrO}_4)_3 \\ (\text{H}_2\text{O})_3 \end{smallmatrix} \right]$.

Chromates and sulphates, as is well known, behave chemically alike in many respects. Some chromates and sulphates are isomorphous and form mixed crystals. As chromium sulphates are known to form a well defined series of complex salts, it was considered worthwhile to investigate if similar chromium chromates might be prepared. Normal chromic chromate like normal and simple chromic sulphate is unknown. A number of oxides, such as Cr_5O_8 , CrO_4 , Cr_5O_{12} etc., with composition intermediate between chromic oxides, Cr_2O_3 , and chromic anhydride, CrO_3 , have been described in literature as chromium chromates. All these compounds arise either by partial oxidation of chromic hydroxide or by partial reduction of chromium trioxide, or by the interaction of chromic salts and chromates.

Brown coloured Cr_5O_8 , or $2\text{Cr}_2\text{O}_3 \cdot \text{CrO}_3$, was prepared by heating chromyl chloride vapour or chromium trioxide to 300° (Wohler and Néger, *Ann. chim. phys.*, 1859, *iii*, **56**, 501). A hydrated variety, $2\text{Cr}_2\text{O}_3 \cdot \text{CrO}_3 \cdot 9\text{H}_2\text{O}$ was described by Popp (*Annalen*, 1890, **166**, 93), who prepared it by boiling a mixture of potassium dichromate and sodium thiosulphate solution. The brown compound, $2\text{Cr}_2\text{O}_3 \cdot \text{CrO}_3$ was also obtained by Le Blanc by mixing solutions of neutral chromate and tervalent chromium salt (*Ann. chim. phys.*, 1926, *x*, **6**, 182). Anhydrous chromium dioxide CrO_2 or $\text{Cr}_2\text{O}_3 \cdot \text{CrO}_3$ was prepared by evaporating the solution of Cr_2O_3 in nitric acid and heating the residue to 290° (Jonitschwitsch, *Helv. Chim. Acta*, 1920, **3**, 40). This and the previous compound are regarded as basic chromium chromate. Normal chromic chromate, Cr_5O_{12} or $\text{Cr}_2\text{O}_3 \cdot 3\text{CrO}_3$, or $\text{Cr}_2(\text{CrO}_4)_3$ was prepared by heating CrO_3 to 250° (Traube, *Annalen*, 1848, **66**, 87; Rothaug, *Z. anorg. Chem.*, 1913, **84**, 165; Simon and Schmidt, *ibid.*, 1924, **152**, 191). It was converted into a soluble modification by keeping in contact with water for a long time.

Paneth mentions that if an excess of silver chromate is treated with a solution of chromic chloride, the colour of the solution turns reddish brown. On evaporating the filtrate from silver chloride a dark brown lustrous colloidal solid or varnish is left. This, on analysis, gave a ratio of Cr^{III} and Cr^{VI} as 2:3 (Paneth, "Radioelements as Indicators", 1928, p. 48, footnote). With a view to study the nature and composition of the product thus cursorily reported and to test the validity of the conclusions arrived at by previous workers on the subject, the present investigation was undertaken.

The filtrate from the double decomposition of silver chromate and chromic chloride solution was evaporated to dryness in vacuum over concentrated H_2SO_4 . The product, which is soluble in water, was examined both by chemical and physical methods. Measurement of conductivity of its aqueous solution, determination of its molecular weight and magnetic susceptibility as well as a qualitative study of its absorption spectra seem to suggest that the substance is not a simple chromate, $\text{Cr}_2(\text{CrO}_4)_3 \cdot \text{XH}_2\text{O}$, but contains a complex chromato-chromiate anion besides the simple Cr^{3+} cation. Its constitution should, therefore be represented by the formula



In the latter case the complex is represented as a triaquo-trichromato-chromiate anion with the chromate group occupying only one co-ordination position. This is also supported by the general chemical behaviour of the substance.

EXPERIMENTAL.

Freshly prepared pure silver chromate in excess was triturated in a mortar with a concentrated solution of chromic chloride. The deep brown filtrate from silver chloride and excess of silver chromate on evaporation in vacuum over concentrated H_2SO_4 gave a dark brown glassy mass. The latter on analysis gave a ratio between Cr^{III} and Cr^{VI} as 2:3. Chromatic chromium (Cr^{VI}) was determined by iodimetry. Total chromium was also estimated similarly after fusion with sodium peroxide. From the two results chromium present as Cr^{III} was calculated.

The solid substance was digested with glacial acetic acid several times at the room temperature. The solid was then dried in vacuum over concentrated H_2SO_4 and solid KOH to a constant weight. The dry substance was analysed. (Found CrO_3 , 59.75; Cr_2O_3 , 29.96. $\text{Cr}_2\text{O}_3 \cdot 3\text{CrO}_3 \cdot 3\text{H}_2\text{O}$ requires CrO_3 , 59.50; Cr_2O_3 , 30.0 per cent).

Cryoscopic Measurement.

Measurement of the molecular weight of the substance in aqueous solution by the freezing point method gave the following results.

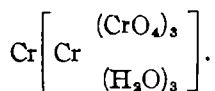
Substance g/100 c.c. (on anhydrous basis).	Depression Δ ($^{\circ}$ C)	Mol. wt. (<i>m</i> .)	Vant Hoff's factor ($i = M/m$).
2.4530	0.21	210.3	2.141
1.2265	0.12	184.05	2.401
0.61325	0.063	169.82	2.66

The results are more in favour of the constitution $\text{Cr}[\text{Cr}(\text{CrO}_4)_3]$ or $\text{Cr} \left[\begin{smallmatrix} (\text{CrO}_4)_3 \\ (\text{H}_2\text{O})_3 \end{smallmatrix} \right]$, which should dissociate into two ions, than of $\text{Cr}_2(\text{CrO}_4)_3$,

which would dissociate into five ions. Partial dissociation of the complex chromato-chromiate or aquochromato-chromiate anion, increasing with dilution, is also indicated.

Equivalent Conductivity at 25 $^{\circ}$.

The above conclusion is supported also by the measurement of the equivalent conductivity of solutions of the substance, assuming it to be a binary ter-tervalent electrolyte based on the formula $\text{Cr}[\text{Cr}(\text{CrO}_4)_3] \cdot 3\text{H}_2\text{O}$ or



$v(\text{dilution})$...	32	64	128	256	512	1024
λ , ...	65.51	72.40	80.89	87.10	88.69	89.86

Measurement of p_H value in N/15-solution of the Substance.

An N/15-solution of the substance was prepared containing 1/90th of the formula weight of the substance per litre. p_H value of this solution and of an equivalent solution of chromic acid as well as of N/10-chromic chloride and hydrochloric acid were measured at room temperature by means of glass electrode for the purpose of comparison. The results are given below.

	N/10- CrCl_3	N/10-HCl	N/15- H_2CrO_4	N/15-Substance
p_H	1.25	1.04	1.82	2.87

The p_H of the solution of the substance is much higher than that of an equivalent solution of chromic acid which would not have been the case if it were simply normal chromic chromate, $\text{Cr}_2(\text{CrO}_4)_3$, as the latter, representing a salt of a very weak base and a weak acid, would have hydrolysed more or less completely in solution giving a p_H value almost equal to that of chromic acid. The p_H value of $N/10$ - CrCl_3 is found to approach more closely that of hydrochloric acid of the same equivalent strength though HCl is much stronger acid than H_2CrO_4 .

Magnetic Susceptibility.

The susceptibility of the substance was determined in a Curie's balance at 30° .

$\chi_m = 18.95 \times 10^{-6}$; $\chi_m = 506 \times 18.95 \times 10^{-6} = 9588.7 \times 10^{-6}$. From which $\chi_a = 1942.72 \times 10^6$, and $\mu_{\text{Weiss}} = 10.79$ per g. atom of Cr .

This is more or less in agreement with the value that might be deduced

either for $\text{Cr}_2(\text{CrO}_4)_3 \cdot 3\text{H}_2\text{O}$ or $\text{Cr} \left[\begin{array}{c} (\text{CrO}_4)_3 \\ \text{Cr} \\ (\text{H}_2\text{O})_3 \end{array} \right]$, which should be 8.9, as

chromium, either as a simple or a complex trivalent ion, has got the same magnetic moment of 19.0 Weiss magneton, and a moment of 2.2 Weiss magneton in the form of CrO_4^{3-} anion.

Absorption Spectra.

The absorption spectra of the substance in aqueous solution is entirely different from those of chromic chloride, chromic acid, potassium chromate and dichromate. Solutions having the same theoretical concentration of Cr^{3+} ions for the chromic chloride and the substance were employed for comparison, as also the solutions having the same theoretical concentration of CrO_4^{3-} ions for the substance, chromic acid, potassium chromate and dichromate. The difference is evident from the following Plates I and II.

The solution of the substance was made on the assumption that it was a simple chromic chromate, $\text{Cr}_2(\text{CrO}_4)_3 \cdot 3\text{H}_2\text{O}$. Time of exposure (15 sec.) and the thickness of the solutions (1 cm.) in the cell were kept constant in all cases. The strength of the chromic chloride solution employed was $N/10$, the strength of the solution of the substance was adjusted accordingly.

The strengths of the solutions of chromic acid, potassium chromate and dichromate were so adjusted that the concentration of Cr^{VI} in them was equal to that of the chromic chromate solution. A glance at the absorption spectra in the above plates shows that the substance possesses a strong general absorption in the visible from 5750 Å onwards and also a general absorption towards the extreme red beyond 6600 Å. In other words, the substance in aqueous solution shows only a limited range of transmission between 5750—6600 Å in the visible.

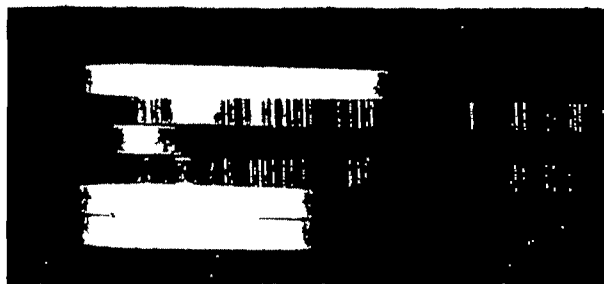
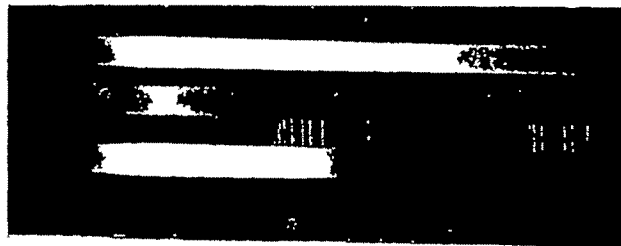
It may, therefore, be concluded that the solution of the substance must at least contain an ion other than Cr^{+++} and CrO_4^{--} or $\text{Cr}_2\text{O}_7^{--}$.

The substance also shows some characteristic chemical behaviour. It dissolves very slowly in cold water but more rapidly in hot water. The solution gives a brownish precipitate on boiling. A dilute solution of the substance gives no precipitate in the cold with silver nitrate solution, but precipitates red silver chromate with excess of the reagent. Solutions of barium chloride and lead acetate give a brown precipitate. Aqueous ammonia decomposes it with precipitation of chromic hydroxide.

My grateful thanks are due to Prof. P. Rây, who suggested this piece of work, for his kind guidance and advice during the course of this investigation, as well as for all the facilities of working in his laboratory.

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Received September 25, 1939



VITAMIN C AND TOXINS. PART IV. THE EFFECT OF TETANUS TOXIN ON VITAMIN C METABOLISM.

BY BAIDYANATH GHOSH.

The effect of the injection of 0.5 m.l.d. of tetanus toxin caused a decrease in true ascorbic acid value in the blood, liver, kidney and adrenal tissues of guinea-pigs. The urinary excretion of free ascorbic acid was lowered during toxic condition with simultaneous increase in combined ascorbic acid.

A comparative study was carried out for the estimation of combined ascorbic acid in the urine by the method of Scarborough and Stewart and that of Sen-Gupta and Guha.

Recently it has been observed (Ghosh, *J. Indian Chem. Soc.*, 1939, **16**, 241) that in guinea-pigs injection of sublethal doses of standardised diphtheria toxin caused an appreciable decrease of ascorbic acid content of blood, adrenal, liver and kidney. Similar decrease in the urinary excretion of free ascorbic acid was observed. Simultaneously, the amount of combined ascorbic acid also excreted in the urine during toxic condition was found to be always greater than that usually excreted by the same guinea pig in the normal state of health. The present investigation of the effect of tetanus toxin was undertaken in order to test the previous view that during the period of infection or disease, the blood, urine and tissues like adrenal, liver and kidney suffer a loss of ascorbic and that a part of this lost ascorbic acid is eliminated in the urine in a combined state. Recently, Kaiser and Slavin (*J. Pediat.*, 1938, **13**, 322) have shown a significant correlation between low blood ascorbic acid values and severe infection with hemolytic streptococci. Thus ascorbic acid seems to play an important part in the defence mechanism of the body as it is always affected during the period of infection or intoxication.

The method for the estimation of combined ascorbic acid in the urine which was described in the previous communication, was that of Scarborough and Stewart (*Biochem. J.*, 1937, **31**, 2231). In this paper the method described by Sen-Gupta and Guha (*J. Indian Chem. Soc.*, 1937, **14**, 95; Sen-Gupta and Guha, *Science and Culture*, 1938, **3**, 398; Guha and Sen-

Gupta, *Nature*, 1938, **141**, 974) is adopted for the estimation of combined ascorbic acid. A comparative study of these two methods has been carried out simultaneously with the same sample of urine and it is found that the method of Sen-Gupta and Guha can serve for the purpose and it can be done in a shorter interval of time.

EXPERIMENTAL.

Guinea-pigs weighing between 250 and 350 g. were kept on a ration consisting of green grass and germinated gram. Five groups of guinea-pigs were taken, each group containing 5 animals. One group served as control while the animals of the other four groups were injected with 0.5 m.l.d. of standardised tetanus toxin. The blood was drawn out from the heart of guinea-pigs at intervals of 24, 48, 72 and 96 hours. The ascorbic acid content was determined from 2 c.c. of oxalated blood, after precipitation of proteins by trichloroacetic acid, by the usual titration with 2:6-dichlorophenol-indophenol (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935, **12**, 30). The results are given in Table I.

TABLE I.

No. of expt.	Mg. of ascorbic acid per 100 c.c. of blood				
	Hours after injection of 0.5 m.l.d. of tetanus toxin.				
	Normal	24	48	72	96
1	0.90	1.00	0.94	0.55	0.55
2	0.96	0.75	0.80	0.54	0.38
3	0.73	0.95	0.53	0.45	0.37
4	1.11	0.89	0.63	0.47	0.43
5	0.75	0.98	0.58	0.58	0.38
Mean	0.89	0.91	0.69	0.50	0.42

A progressive decrease in blood ascorbic acid was observed after 48 hours of toxin injection.

Similar experiments were carried out with other groups of guinea-pigs in order to determine the true ascorbic acid value of the adrenal, liver and kidney 24, 48, 72 and 96 hours after the injection of 0.5 m.l.d. of tetanus toxin (Table II). True ascorbic acid value was determined by the enzymic oxidation (with ascorbic acid oxidase) of an aliquot of the tissue filtrate after

the estimation of the total reducing substances present (Sen-Gupta and Guha, *Science and Culture*, 1938, 3, 398; Guha and Sen-Gupta, *Nature*, 1938, 141, 974).

TABLE II.

A. *Adrenal.*

Mg. of ascorbic acid per g. of adrenal.
Hours after 0.5 m.l.d. of tetanus toxin injection.

No. of expt.	Normal	24	48	72	96
1	0.432	0.366	0.352	0.295	0.276
2	0.407	0.357	0.430	0.258	0.312
3	0.380	0.370	0.430	0.273	0.300
4	0.453	0.505	0.478	0.367	0.206
5	0.452	0.500	0.475	0.476	0.330
Mean	0.428	0.419	0.441	0.333	0.284

B. *Liver.*

Mg. of ascorbic acid per 5g. of liver.
Hours after 0.5 m.l.d. of tetanus toxin injection.

No. of expt.	Normal	24	48	72	96
1	0.870	0.866	0.838	0.650	0.452
2	0.637	0.706	0.510	0.567	0.450
3	0.694	0.591	0.584	0.589	0.608
4	0.577	0.529	0.670	0.390	0.562
5	0.690	0.890	0.593	0.644	0.526
Mean	0.696	0.711	0.639	0.568	0.519

C. *Kidney.*

No. of expt.	Mg. of ascorbic acid per g. of kidney.				
	Hours after 0.5 m.l.d. of tetanus toxin injection.				
	Normal	24	48	72	96
1	0.136	0.116	0.115	0.111	0.073
2	0.093	0.086	0.077	0.090	0.087
3	0.060	0.062	0.077	0.104	0.096
4	0.087	0.064	0.084	0.064	0.076
5	0.112	0.094	0.072	0.066	0.053
Mean	0.097	0.092	0.084	0.087	0.077

The amount of combined ascorbic acid in the urine excreted by a guinea-pig during normal and toxic conditions was determined by the method of Scarborough and Stewart (*loc. cit.*) as previously described by Ghosh (*loc. cit.*). A comparative study of the values obtained by the method of Scarborough and Stewart (*loc. cit.*) and that of Guha and Sen-Gupta (*Nature*, 1938, **141**, 974; Sen-Gupta and Guha, *loc. cit.*) was made from the same sample of urine (*vide* Table III). An aliquot (20 c.c.) of the total acidified urine (50 c.c.), obtained after the removal of the thiosulphate by barium acetate method, was taken in a conical flask (100 c.c.) into which H_2S was allowed to bubble for 5 minutes. Then the flask, while H_2S was being made to bubble, was immersed in a boiling water-bath and the contents were heated for 15 minutes after which the flask was placed in a cold water-bath. When the flask was cold bubbling of H_2S was stopped and H_2S was then removed by a current of CO_2 or coal gas (previously bubbled through dichromate and sulphuric acid mixture*). The urine after the removal of H_2S was made up to the original volume (20 c.c.) by the addition of distilled water and an aliquot of it was titrated with the dye. The remaining portion was allowed to undergo enzymic oxidation in order to estimate the true ascorbic acid value. The values of ascorbic acid content (both free and combined) of the urine measured by this method of Sen-Gupta and Guha are given in Table IV. The ascorbic acid content (both free and combined) of the urine was estimated for three or four consecutive days before and after the injection of tetanus toxin into the same guinea-pig. The results are given in Table III.

* It has been found that coal gas, when purified by bubbling through chromic acid mixture, can be used to remove H_2S completely without affecting the ascorbic acid in the solution.

TABLE III.

Mg. of ascorbic acid excreted in the urine per animal during 24 hours.

Combined ascorbic acid in terms of ascorbic acid

No. of expt	No. of days.	Free ascorbic acid.		Scarborough & Stewart.		Sen-Gupta & Guha.	
		Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection.
I	1	0.331	0.224	0.162	0.206	0.114	0.143
	2	0.280	0.148	0.108	0.269	0.085	0.259
	3	0.251	0.205	0.131	0.115	0.009	0.151
	4	0.280	0.214	0.160	0.174	0.070	0.176
	Mean	0.285	0.197	0.140	0.191	0.069	0.182
II	1	0.332	0.249	0.192	0.241	0.107	0.139
	2	0.332	0.201	0.210	0.240	0.112	0.196
	3	0.332	0.185	0.148	0.196	0.048	0.195
	4	0.332	0.206	0.210	0.283	0.049	0.251
	Mean	0.332	0.210	0.190	0.240	0.097	0.195
III	1	0.134	0.158	0.130	0.135	0.039	0.029
	2	0.202	0.132	0.123	0.075	0.038	0.077
	3	0.243	0.100	0.090	0.247	0.040	0.123
	4	0.218	0.100	0.043	0.300	0.010	0.094
	Mean	0.199	0.122	0.096	0.189	0.032	0.080
IV	1	0.131	0.162	0.232	0.215	0.016	0.043
	2	0.178	0.162	0.111	0.105	0.005	0.000
	3	0.246	0.125	0.054	0.250	0.037	0.086
	4	0.150	0.136	0.059	0.270	0.044	0.065
	Mean	0.176	0.146	0.114	0.210	0.025	0.048
V	1	0.134	0.131	0.091	0.233	0.000	0.087
	2	0.239	0.179	0.094	0.241	0.066	0.129
	3	0.170	0.158	0.074	0.248	0.024	0.097
	4	0.150	0.158	0.109	0.222	0.170	0.169
	Mean	0.173	0.156	0.092	0.236	0.065	0.095
VI	1	0.174	0.110	0.097	0.298	0.000	0.054
	2	0.158	0.162	0.030	0.109	0.016	0.163
	3	0.174	0.162	0.093	0.176	0.044	0.098
	Mean	0.168	0.144	0.067	0.194	0.020	0.105

Another set of experiments was carried out with guinea-pigs injected with 0.5 m. l. of tetanus toxin and the results obtained by Sen-Gupta and Guha's method are shown in Table IV.

TABLE IV.

Injected with 0.5 m. l. d. of tetanus toxin.

Mg. of ascorbic acid excreted in the urine during 24 hours per guinea-pig.					
No. of Expt.	No. of days.	Free ascorbic acid.		Combined ascorbic acid in terms of ascorbic acid (Sen-Gupta & Guha)	
		Before toxin injection.	After toxin injection.	Before toxin injection.	After toxin injection.
I	1	0.218	0.221	0.006	0.163
	2	0.260	0.148	0.230	0.107
	3	0.270	0.128	0.120	0.190
	Mean	0.249	0.163	0.118	0.153
II	1	0.473	0.347	0.049	0.143
	2	0.350	0.290	0.139	0.170
	3	0.314	0.221	0.089	0.090
	4	0.347	0.221	0.143	0.091
	Mean	0.320	0.269	0.103	0.123
III	1	0.252	0.185	0.038	0.175
	2	0.286	0.215	0.164	0.093
	3	0.260	0.217	0.100	0.038
	4	0.280	0.221	0.070	0.191
	Mean	0.269	0.202	0.094	0.124
IV	1	0.270	0.228	0.050	0.041
	2	0.224	0.138	0.056	0.124
	3	0.225	0.257	0.098	0.150
	4	0.246	0.224	0.046	0.126
	Mean	0.241	0.211	0.062	0.110

DISCUSSION.

The injection of a sublethal dose of tetanus toxin into guinea-pigs has been found to produce a diminution of the free ascorbic acid content of blood, liver, kidney, adrenal and urine. At the same time urine in the infected condition appeared to contain a considerably increased amount of combined ascorbic acid, as estimated by the methods of Sen-Gupta and Guha (*loc. cit.*) and of Scarborough and Stewart (*loc. cit.*). By the latter method the values obtained are greater, as the treatment with H_2S is more prolonged. But in both methods, ascorbic acid oxidase was used and differences in the combined ascorbic acid content of the urine between the normal and toxic conditions were noticeable.

My best thanks are due to Prof. B. C. Guha for his advice and encouragement. Our thanks are due to the Indian Research Fund Association for financing these researches.

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Received September 4, 1939

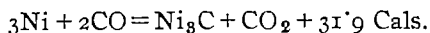
CATALYTIC FORMATION OF METHANE FROM CARBON MONOXIDE AND HYDROGEN. PART VI. ON THE POISONING BY CARBON DEPOSITION.

BY K. M. CHAKRAVARTY.

It has been shown that the reaction $2\text{CO} = \text{C} + \text{CO}_2$ is catalysed both by nickel and nickel carbide. The reaction $\text{CO} + 3\text{H}_2 = \text{CH}_4 + \text{H}_2\text{O}$ is accelerated by nickel. Poisoning in both the cases is mainly due to the deposited carbon adsorbed on the surface. In the latter case the carbon deposition is due to reaction $\text{CO} + \text{H}_2 = \text{C} + \text{H}_2\text{O}$ as well.

The fact that nickel catalysts lose activity due to carbon deposition is well known. The deposited carbon may be absorbed on the nickel particles or may simply form a layer on the catalytic surface. In the process of carbon deposition, nickel may also undergo carbide formation. The point therefore arises as to how far each of these affects the activity of the catalyst and whether nickel carbide also has any catalytic influence.

Ruff and co-workers (*Metallurgie*, 1912, 9, 143; *Z. anorg. allgem. Chem.*, 1914, 88, 386) have found that in molten nickel carbon is dissolved to the extent required by the formula Ni_3C . This solubility remains constant till the boiling point 2075° is reached showing thereby that a carbide is formed. Recent investigations (Meyer and Scheffer, *Rec. trav. chim.*, 1927, 46, 1; Bahr and Bahr, *Ber.*, 1928, 61, 2177, 1930, 63, 99; Tutiya, *Bull. Inst. Phys. Chem. Res. Japan*, 1931, 10, 951; Schmidt, *Z. anorg. allgem. Chem.*, 1933, 216, 83; Jacobsen and Westgren, *Z. physikal. Chem.*, 1933, B20, 361; Hedvali and Sandford, *ibid.*, 1935, B29, 455) have shown that this carbide is also formed when carbon monoxide is passed over reduced nickel at about 260° . No carbide is, however, formed at 700° (Meyer and Scheffer, *loc. cit.*) and at 500° only 2% of the total carbon deposited combined with nickel (Bahr and Bahr, *loc. cit.*). This shows the exothermic nature of the reaction. It has been represented by Bahr and Bahr by the equation



According to Tutiya the heat of reaction is 46.4 Cals.

This formation of the carbide has been definitely established both by physical and chemical tests. Complete solution of the product in hydrochloric acid giving hydrocarbon gases indicated clearly the absence of free carbon in the sample and the formation of carbide. The magnetic property indicated the absence of free nickel. The estimation of carbon and nickel in

the sample has established that a carbide of the formula Ni_3C was formed. The X-ray diagram which was different from that of carbon and nickel also proved that a definite compound was obtained

The heat of reaction whereby carbon combines with nickel to yield nickel carbide has recently been redetermined by Roth (*Arch. Eisenhüttenw.*, 1929-30, 3, 339; *Stahl. u. Eisen*, 1929, 49, 1763) giving the figure -9.2 ($\pm 10\%$) Cals. This shows the endothermic nature of the reaction in question and explains why a nickel carbon solution containing 6.37% of carbon (Ni_3C) on being suddenly cooled by dropping into cold water did not show any line for carbide in an X-ray diagram. The extent of stability of this carbide formed at a much lower temperature has therefore been examined by Bahr and Bahr (*loc. cit.*). On heating the carbide in an atmosphere of nitrogen above 380-400°, liberation of free carbon has been detected by the action of hydrochloric acid. The decomposition temperature, however, depends upon the condition of preparation. (Bahr and Bahr, *loc. cit.*). A sample has been found to be unaffected even at 500° when heated for 14 hours. Generally, however, these carbide preparations are stable up to 400°. This stability up to a temperature of 400° in an atmosphere of nitrogen of the nickel carbide, formed by the action of carbon monoxide upon nickel, suggests that it will show no tendency to decompose at least at 400° in an atmosphere of carbon monoxide.

The investigation of Cleminson and Briscoe (*J. Chem. Soc.*, 1923, 123, 2148) has revealed that carbon monoxide does not dissociate at or below 400° when glass forms the contact material. From Bahr and Bahr's paper it is also clear that the fission of carbon monoxide does not take place at 250°-270° on a nickel carbide surface. From their paper it is also true that the carbide formation must have been complete after 20 hours at temperatures higher than 270°. Since the deposition of carbon continued even after 90 hours, it is evident that the nickel carbide formed was catalysing the reaction in question at temperatures higher than 270°, the catalytic effect of glass being negligible below 400°.

The question arises as to whether nickel also can act as a catalyst for this reaction in the temperature range in which the carbide is stable. The fact that nickel can catalyse the carbon deposition at temperatures higher than those at which the carbide decomposes, indicates that it will also perform the same operation in the lower temperature range as well, provided a suitable nickel preparation can be obtained which will not be converted into the carbide in the temperature region in question. Hedvall and Sandford (*loc. cit.*) have succeeded in preparing such a nickel catalyst. On keeping their catalyst at 260° for 460 hours in a current of carbon monoxide, 1 c.c.,

per minute only, 3% of the nickel were converted into the carbide. When tried at 350° for 190 hours there was carbon deposition but no carbide was practically formed. But the fact that the reaction $2\text{CO} = \text{C} + \text{CO}_2$ was continually catalysed by this nickel preparation is a clear indication of the reaction being catalysed by nickel from 260°-360°. According to Cleminson and Briscoe (*loc. cit.*) this reaction just begins at 360° on purified sugar charcoal. Therefore, the catalytic action of deposited carbon, if any, will be negligible. It was also noticed that the reaction rate suddenly increased at the Curie points of the original nickel catalysts. This lent additional support to the view that in experiments of Hedvall and Sandford nickel and not the carbide was the main catalyser; otherwise these temperatures would have widely differed. Their figures for the Curie points etc. are quoted below.

Catalyst	Curie point	• Temperature of sudden change in the reaction rate
Ni (ii)	354-359°	354-359°
Ni (iii)	364-370°	364-370°

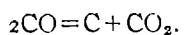
Taylor (*J. Amer. Chem. Soc.*, 1931, **53**, 593) has found that at about 270° the adsorption of carbon monoxide on nickel catalyst is irreversible presumably being attached to the nickel surface as Ni_3C and NiO . At lower temperature the sorption is reversible probably due to the formation of adsorption compounds or to van der Waals absorption. Since particles of different degrees of activity exist on a nickel catalyst surface, it is impossible that in the temperature range 240°-300° for example, the retention of carbon will be mainly due to the formation of both nickel carbide and adsorption compounds. As a matter of fact, Horiba and Ri (*Bull. Chem. Soc. Japan*, 1928, **3**, 18; *Rec. trav. chim.*, 1932, **51**, 641) have shown that the retardation of the reaction



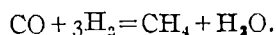
on the nickel surface is initially due to absorption of carbon monoxide but from 240°-300° it is due to the formation of nickel carbide and adsorption compound. But since a nickel surface (Bahr and Bahr, *loc. cit.*) continues to catalyse the reaction even when it is completely converted into carbide suggests that adsorbed carbon is the main poison. Bhatnagar and co-workers (*Indian J. Phys.*, 1929, **3**, 53) have proved that chemical forces are involved in this process of adsorption. They have noticed that the paramagnetic nickel becomes diamagnetic on being adsorbed on carbon particles. So due to adsorption the active nickel particles are not only covered with carbon

particles but also their unsaturation is lowered. As a consequence the poisoning due to adsorption will be more pronounced in comparison with that due to the carbide formation. From Meyer and Scheffer's paper (*Rec. trav. chim.*, 1927, **46**, 1) the inert nature of the carbon nickel mixture obtained by the action of carbon monoxide on nickel at 700° is evident, for like carbon the product was unaffected by the action of hydrochloric acid while both nickel and its carbide are readily soluble.

It, therefore, appears clear that both nickel and its carbide can catalyse the reaction



According to Horiba and Ri (*loc. cit.*) the poisoning is partly due to carbide formation. So the activity of the carbide surface produced from a nickel one will have a comparatively low activity. But adsorbed carbon is mainly responsible for poisoning. More or less similar conclusions can also be drawn for the catalysis of the reaction by a nickel catalyst.



Sabatier and Senderens (Sabatier and Reid, "Catalysis in Organic Chemistry," 1923, p. 144) noticed that while catalysing the above reaction at 250° using as reactants one part by volume of carbon monoxide and three parts by volume of hydrogen, the nickel catalyst was slightly carburised. The complete solubility of this product in hydrochloric acid indicated that nickel carbide was formed and no free carbon was deposited. The fact that the activity of the catalyst was not appreciably altered due to carbide formation shows that the formation of carbide to a small extent, which is realisable when the reaction is carried on below 250° , does not affect the activity to an appreciable degree. But when carried out above 250° , the carbide formation becomes more and more considerable and has a deliterious effect upon the catalyst. Under the above circumstances, however, the activity remains undiminished when a 5 : 1 mixture instead of a 3 : 1 of hydrogen and carbon monoxide is used. Evidently this is due to the diluent effect of hydrogen which leads to a retardation of the reaction.

An analysis of the experimental data of Litkenhous and Mann (*Ind. Eng. Chem.*, 1937, **29**, 934) throws more light in this connection. In the following table are given the logarithms of the equilibrium constants of

a number of reactions calculated from their experimental data. They passed a mixture of nickel carbonyl vapour and hydrogen through the reaction chamber at atmospheric and higher pressures. The composition of the initial gas mixture and the rate of passing were the same throughout the whole series of experiments.

TABLE I.

Reaction	at 350°.	at 400°.
1. $2\text{CO} + 2\text{H}_2 = \text{CH}_4 + \text{C}(\text{O})$	(1) 7.0166	(1) 5.4124
	(2) 6.5392	(2) 5.0308
$K_p = \frac{p_{\text{CH}_4} \times p_{\text{CO}_2}}{p_{\text{CO}}^2 \times p_{\text{H}_2}^2}$	(3) 7.1225	(3) 3.4178
	6.4120	3.4268
	6.9462	3.4537
		3.4401
2. $\text{CO} + 3\text{H}_2 = \text{CH}_4 + \text{H}_2\text{O}$	(1) 5.7040	(1) 4.3385
	(2) 5.2781	(2) 3.9862
$K_p = \frac{p_{\text{CH}_4} \times p_{\text{H}_2\text{O}}}{p_{\text{CO}} \times p_{\text{H}_2}^3}$	(3) 4.7426	(3) 3.2748
	5.1498	3.3109
	5.6032	3.3868
		3.3888
3. $2\text{CO} = \text{C} + \text{CO}_2$	(1) 5.2330	(1) 4.1619
	(2) 4.8700	(2) 3.8510
$K_p = \frac{p_{\text{CO}_2}}{p_{\text{CO}}^2}$	(3) 5.3475	(3) 2.2169
	4.8343	2.1776
	5.2284	2.1725
		2.2003

(1) Chipman, *Ind. Eng. Chem.*, 1932, **24**, 1013.

(2) Reinders, *Z. physikal. Chem.*, 1927, **130**, 405.

(3) Litkenhous & Mann, *Ind. Eng. Chem.*, 1937, **29**, 934.

TABLE I (contd.).

4. $\text{H}_2\text{O} + \text{CO} = \text{CO}_2 + \text{H}_2$	(1) 1.3126	(1) 1.0739
$K_p = \frac{p_{\text{CO}_2} \times p_{\text{H}_2}}{p_{\text{H}_2\text{O}} \times p_{\text{CO}}}$	(2) 1.2611	(2) 1.0446
	(3) 1.3783	(3) 0.1433
	1.2624	0.1156
	1.3653	0.1361
		0.1024
5. $\text{CO} + \text{H}_2 = \text{C} + \text{H}_2\text{O}$	(1) 3.9203	(1) 3.0877
$K_p = \frac{p_{\text{H}_2\text{O}}}{p_{\text{CO}} \times p_{\text{H}_2}}$	(2) 3.6089	(2) 3.8664
	(3) 3.9678	(3) 2.0738
	3.5719	2.0620
	3.8630	2.0362
		2.0979

From the above table one finds curiously that though all the reactions generally reached equilibrium at 350° , practically none of them reached that stage at 400° . Evidently the nickel particles were seriously poisoned at 400° . There was carbon deposition in all the experiments from 240° to 400° and as a matter of fact the deposition was the greatest at the lowest temperature *viz.* 250° and the least at the highest temperature *viz.* 400° . That this was so will be clear from Table II and is in accordance with the thermodynamic requirement.

TABLE II.

Temp.	250°	300°	350°	400°
C deposited (mole)	0.0001057	0.0000969	0.0000885	0.0000867

So it is evident that poisoning was not proportional to the amount of carbon deposition but depended rather on how the deposition occurred. It has been concluded before that nickel carbide becomes unstable above 400° . The nickel preparation of Hedvall and Sandford (*loc. cit.*) did not yield the nickel carbide at 360° though there was carbon deposition on passing carbon monoxide over it. Bahr and Bahr (*loc. cit.*) have shown that the combined carbon is readily converted into methane at 180 – 380° by the action of hydrogen, whereas free carbon remains unchanged under these conditions. Their gas analysis data are as CO_2 , 0.3; S.Kw, 0.2; O_2 , 0.0; CO , 0.2; H_2 , 2.4; CH_4 , 28.6; C_2H_6 , 3.2; and N_2 , 65.1 (als Spulgas) Total, 100.0.

It therefore follows that at 400° and higher temperatures the carbide formation will be more or less impossible where the reacting gas instead of being only carbon monoxide is a mixture of this gas with hydrogen. At lower temperatures, however, the carbon deposition producing nickel carbide will increase gradually while the free carbon deposition will gradually decrease. So, it is clear from the data of Litkenhous and Mann given above that carbon deposition which leads to carbide formation does not affect the activity of nickel catalyst for the reaction in question to an extent as free carbon deposition even in smaller doses does. Here the temperature being 400° the question of sintering also comes in. But our experiments with simple nickel catalyst described in a previous paper (*Z. Elektrochem.*, 1931, 37, 775) show that this effect is rather small.

Kubokawa (*Rev. Phys. Chem. Japan*, 1938, 12, 40) studied the decomposition of methane on nickel surface at 500°. The activity of the catalyst became constant after it had decreased to certain extent in spite of the accumulation of carbon particles. This shows that the first one or two layers of carbon are responsible for the poisoning and these are evidently the adsorbed carbon layers. The existence of nickel carbide at such a high temperature appears to be impossible. Schmidt (*loc. cit.*) has shown that no carbide is formed by the decomposition of methane over nickel even at 250° though his nickel catalyst was found to yield carbide at 240°-250° by the action of carbon monoxide and at 180°-200° by the action of acetylene.

Eventually one can conclude that for the reaction $\text{CO} + 3\text{H}_2 = \text{CH}_4 + \text{H}_2\text{O}$, nickel is the most active catalyst. Nickel carbide does not poison the catalyst to the extent as adsorbed carbon does. It is also possible in view of Kubokawa's data that unadsorbed carbon particles will not also affect the above reaction to an appreciable extent.

From the behaviour of sugar charcoal-nickel catalysts described in some previous papers (*J. Indian Chem. Soc.* 1925-26, 2, 150, 157; 1927, 4, 431) we have noticed that sintering is checked by sugar carbon acting as a support material and is not a serious poison. The catalysts generally were not highly active. The activity decreased with an increase in the concentration of sugar carbon. Free nickel was present in those catalysts and was indicated by the formation of nickel carbonyl in an atmosphere of of the reacting gases.

But the fact that the deposition of carbon particles on a nickel surface in the process of catalysis affects the activity seriously is partly because

of the fact that the deposition occurs selectively on the most active particles from which the tracks of the reaction on the catalyst surface start and partly due to the fact that under the circumstances the deposition of nickel particles upon the carbon particles is almost impossible.

The carbon deposition which poisons the nickel catalyst has so far been ascribed to the reaction $2\text{CO} = \text{C} + \text{CO}_2$. From our experimental data of a forthcoming paper it is clear that the deposition of carbon may as well be due to the reaction $\text{CO} + \text{H}_2 = \text{C} + \text{H}_2\text{O}$. This can also be inferred from the experimental data of Litkenhous and Mann (*loc. cit.*) and has been dealt with fully in a letter to be published shortly in the Journal of the Industrial & Engineering Chemistry.

I take this opportunity of thanking Prof. J. C. Ghosh, Prof. S. N. Bose and Dr. J. K. Chowdhury for their encouragement.

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Received August 3, 1939.

SPACE GROUP DETERMINATION OF THE CRYSTALS OF MELAMINE.

BY JAGDISH SHANKER, PRABHAKAR N. BALJEKAR AND MATA PRASAD.

The dimensions of the unit cell of the crystal of melamine have been found to be $a = 10.52\text{\AA}$, $b = 7.44\text{\AA}$; $c = 7.33\text{\AA}$. The list of reflecting planes in the crystal shows that the planes (hol) are halved if h is odd and (olo) is also halved. These halvings assign the crystal to the space group C_{2h}^2 . The number of molecules in the unit cell is four.

The crystals of a large number of compounds having one or more benzene rings have been thoroughly studied by X-ray methods. But so far the crystalline structure of only a few substances having a heterocyclic ring of carbon and nitrogen has been completely determined (*cf.* Knaggs, *Proc. Roy. Soc.*, 1935, **A** 160, 576, Robertson, *J. Chem. Soc.*, 1936, 1195; Lansdale, *Z. Kryst.*, 1936, **28**, 471). The present investigation gives the results of space group determination of the crystals of melamine which belongs to the cyanuric series of compounds.

Melamine has been studied crystallographically and the crystals have been found to develop the following faces:—

$$c(001); \quad m(110); \quad q(011); \quad m'(\bar{1}10); \quad \text{and} \quad q(0\bar{1}\bar{1}).$$

The crystals belong to the monoclinic prismatic class. The axial ratio is $a : b : c = 1.4091 : 1 : 0.9783$ and $111^\circ - 47'$ (Groth, "Chemische Kristallographie", p. 564-65).

The crystals of melamine were obtained from water. It was found that generally crystals had curved faces and only a few well developed crystals were obtained. These had developed $c(001)$, $m(110)$ and $m'(110)$ faces, which were identified by measuring the interfacial angles and further confirmed by taking several Laue photographs.

The dimensions of the unit cell calculated from the rotation photographs (Plates, I, II and III) are

$$a = 10.52\text{\AA}; \quad b = 7.44\text{\AA} \quad c = 7.33\text{\AA}.$$

These give the axial ratio $a : b : c = 1.414 : 1 : 0.9851$, which agrees closely with Groth's ratio.

Tables I and II give the list of planes observed in the crystal along with their approximate relative intensities as estimated by the eye.

From Table I, it will be seen that (hol) planes are halved when h is odd and (olo) is also halved. The above halvings assign to the crystal of melamine the space group C_{2h}^2 . The number of molecules calculated from the dimensions of the cell and specific gravity of the crystals (found to be 1.57) is 4 (accurately 3.997).

TABLE I.

Axial planes	Prism planes						
		(h0l)		(0kl)		(hko),	
001	v s.	201	v	011	v s.	110	s
002	s	201	v s	012	w	120	s
003	w m	202	s	013	v w.	130	w.m.
020	v w	202	v s.	021	m s	210	v s.
200	m s	203	s	022	s	230	m
300	w	204	v w	031	v s	400	m.
400	m s	401	s	032	m	310	v s.
		402	s	033	m s	320	s.
				042	m s	330	m s.
						410	m
						420	w m

TABLE II

Plane	Intensity	Plane	Intensity	Plane	Intensity	Plane	Intensity
111	v s	211	v s	311	w	411	m
111	s	211	m	311	m	411	m.
112	s	212	w	312	m s	412	m s
112	m	212	w	313	m s	412	m.
121	m s.	213	w	314	v w	413	m s
121	v s	214	w m	321	m.	421	m s.
122	m	221	v w	321	m s.	421	m s
123	w.	222	v w.	322	w m.	422	m
123	w m	222	v w	322	w.m.	431	w
131	v s	231	w	323	m	511	m.
132	m s	231	v w	331	m	513	m s
132	m	232	v w	332	v s	521	v w
133	m	241	w m	333	w m		
		242	m	341	m		

SHANKER, BAE EKAR & PRASAD

Rotational photograph of melamine

Plate I
a-axis

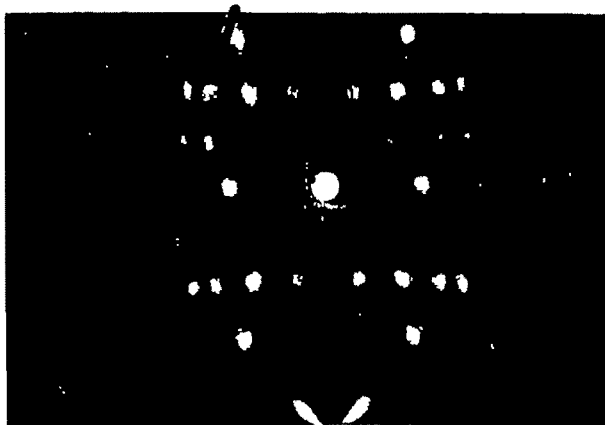


Plate II
b-axis

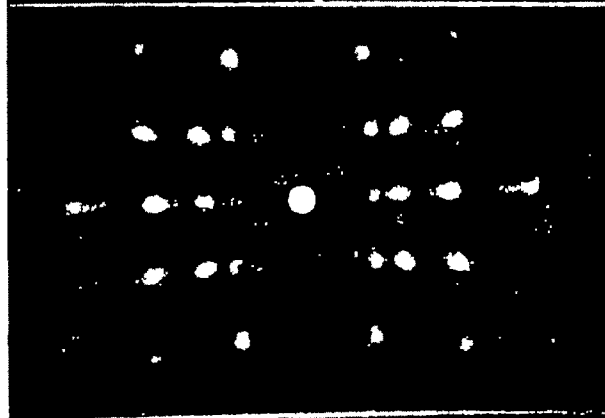
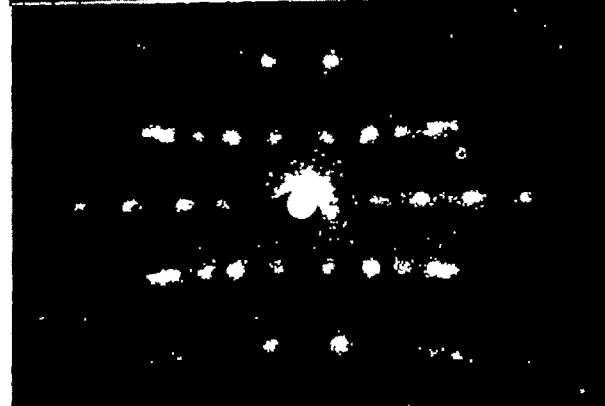


Plate III
c-axis

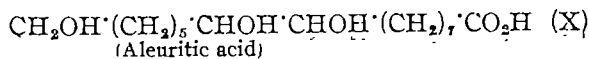
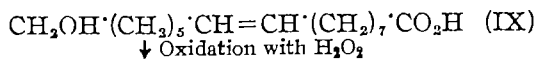
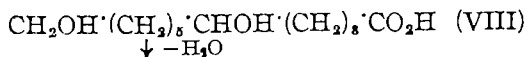
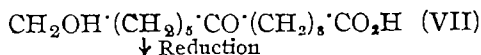
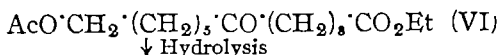
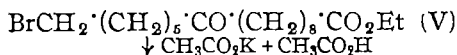
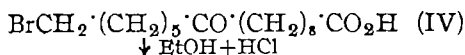
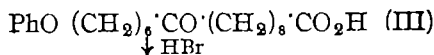
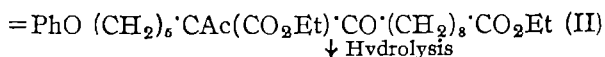
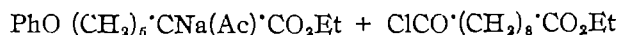
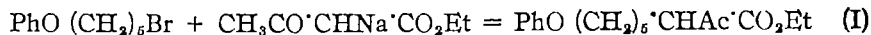


STUDIES IN LONG-CHAIN ACIDS. PART II. ON ALEURITIC ACID (I.)

BY P. C. MITTER AND PHANINDRA CHANDRA DUTTA.

Ethyl 16-acetoxy-10-ketopalmitate has been synthesised as a preliminary to the synthesis of 9:10:16-trihydroxypalmitic acid (aleuritic acid).

Aleuritic acid is one of the important constituents of shellac resin. Its constitution as 9:10:16-trihydroxypalmitic acid has been determined from analytical data, obtained mainly by a study of the products of oxidative degradation (Nagel, *Ber.*, 1927, **60**, 605), but no work has been done to prove the constitution and it seemed, therefore, of interest to attempt a synthesis of the substance along lines closely following that of Robinson and Robinson (*J. Chem. Soc.*, 1925, 175; 1926, 2004; 1930, 745).



ω -Phenoxy-pentamethylene bromide was condensed in the usual way with acetoacetic ester to give (I). Here two molecules of acetoacetic ester were taken to prevent the formation of the bi-molecular condensation product which was always an undesirable residue left in the flask after distillation (*J. Chem. Soc.*, 1915, 898; cf. Robinson, *ibid.*, 1937, 723).

Condensation of this ester with sebacic acid half-ester chloride was carried out in ether solution. The product was directly hydrolysed with dilute alkali. No product was, however, isolated on following the conditions laid down for the hydrolysis of this type of compounds by Robinson and Robinson (*loc. cit.*) and consequently the conditions of Chuang, Tien and Huang (*Ber.*, 1937, 70, 858) were followed. The hydrolysis proceeded slowly in presence of alcohol and it was continuously shaken for 60-70 hours until the whole of neutral matter went into solution. The keto-acid (III), thus obtained, was purified through its sodium salt. Dephenoxylation was carried out by heating with hydrobromic acid and glacial acetic acid for 6 hours (*J. Amer. Chem. Soc.*, 1927, 49, 1829). The yield from this operation was, however, very poor. The bromoketo-acid, thus obtained, was treated with fused potassium acetate in glacial acetic acid and the acetoxy derivative was isolated as the corresponding ethyl ester by treatment with hydrochloric acid and alcohol. The keto group was, however, found to be very inactive so much so that it gave no semicarbazone. Catalytic reduction of this carbonyl group with Adam's PtO_2 catalyst or Pd-BaSO_4 gave no desired result. Reduction of the carbonyl group was also attempted with sodium amalgam in a current of carbon dioxide, but the ester group was knocked off. Similarly there is considerable possibility of the acetyl group being also knocked off along with the ethyl group. Since the yield during dephenoxylation is very poor, we have in mind to proceed with the corresponding methoxy compound, for it has been shown by Robinson (*J. Chem. Soc.*, 1937, 269) that such groups could be easily knocked off with satisfactory yield. Another modification which we are contemplating to introduce in the general scheme, is the reduction of the carbonyl group with aluminium isopropoxide according to Lund (*Ber.*, 1937, 70, 1520). To prepare the methoxy-pentamethylene iodide, we have condensed malonic ester with methoxy-trimethylene iodide. (Robinson, *ibid.*, 1937, 61). ω -Methoxyvaleric ester was reduced with sodium and alcohol. This was converted to chloride by thionyl chloride and pyridine and then to iodide by refluxing with sodium iodide in acetone solution (*J. Chem. Soc.*, 1929, 269).

It has been proved by condensation with acetone that the hydroxyl groups of the natural acid at 9:10 positions occupy *cis*-configuration and

lately it has been shown that the introduction of the two hydroxyl groups with *cis*-configuration at an unsaturation is only possible by oxidation with hydrogen peroxide in presence of OsO_4 (*Ber.*, 1938, 71, 1483).

The work is being continued.

EXPERIMENTAL.

ω-Phenoxy-pentamethylene-acetoacetic Ester (I).—Pentamethylene bromide, prepared from benzoylpiperidine by the application of von Braun's reaction ("Organic Synthesis", Vol. IX, p. 18), was monophenoxyated under conditions identical to those described for the preparation of phenoxytrimethylene bromide. To a well-cooled solution of sodium (1.6 g.) in alcohol (20 c.c.) a mixture of the bromo-ether (16 g.) and acetoacetic ester (17 g., 2 mols.) was added and the solution refluxed on a water-bath for 24 hours. It was poured into water and the oil was extracted with ether, washed with sodium bicarbonate and dried (sodium sulphate). On removal of lower boiling fractions, the ester distilled at $180^\circ/3$ mm., yield 14 g. It gave a brownish violet colouration with ferric chloride in alcoholic solution. (Found : C, 69.3; H, 8.2. $\text{C}_{17}\text{H}_{24}\text{O}_4$ requires C, 69.8; H, 8.2 per cent).

ω-Phenoxy-10-ketopalmitic Acid (III).—Sodium (2.5 g.) was pulverised, covered with dry ether and cooled in ice. To this the above ester (29.2 g.) was slowly added with shaking and on allowing to stand overnight, a clear reddish solution was obtained. To this mixture, the half-ester chloride of sebacic acid was added under cooling. A vigorous reaction set in with immediate separation of sodium chloride. The mixture was allowed to stand overnight, refluxed on the water-bath for 1 hour and was then decomposed with ice and washed thoroughly with sodium bicarbonate to remove the excess of half-ester. On removal of ether, a viscous reddish mass was obtained. This crude condensation product was directly hydrolysed by aqueous alcoholic caustic potash under the following conditions :

25.5 G. of this condensation product were added to 180 c.c. of 4% aqueous caustic potash and the mixture was continuously shaken for 60-72 hours until a clear solution was obtained, traces of neutral matter being removed by ether. It was acidified with ice-cold sulphuric acid and the oil separating was thoroughly extracted with ether, the extract dried over sodium sulphate and the ether removed on the water-bath. After the removal of ether the heating was continued until there was no evolution of carbon dioxide. On cooling, the mass solidified in beautiful crystals. Hydrolysis was completed by again heating this mass with 200 c.c. of 10% caustic soda solution for 2 hours on a water-bath. On cooling, crusts of sodium salts

separated on the surface, which were filtered off and decomposed by boiling with hydrochloric acid. On cooling, the acid crystallised out. It was collected and recrystallised from alcohol in shining plates, m.p. 89° , yield 2 g. (Found : C, 72.93 ; H, 9.3. $C_{22}H_{34}O_4$ requires C, 73.03 ; H, 9.1 per cent).

The keto-ester was prepared by passing a stream of dry hydrogen chloride into a cooled solution of the acid in alcohol and on refluxing for 3 hours on a water-bath on the next day. It distilled at $252^{\circ}/2$ mm., m.p. 50° . (Found : C, 74.0 ; H, 9.7. $C_{24}H_{38}O_4$ requires C, 73.8 ; H, 9.5 per cent).

16-Bromo-10-ketopalmitic Acid (IV).—The keto-acid (10 g.) was placed in a 100 c.c. flask fitted with a long fractionating column and glacial acetic acid (20 c.c.) and constant boiling hydrobromic acid (25 c.c.) were added. The mixture was refluxed over a free flame and allowed to distill off at such a rate that the temperature at the head of the fractionating column was maintained between 115° and 120° . More hydrobromic acid was added during heating. The reaction mixture was heated for 5-6 hours and finally the hydrobromic acid was distilled off as far as possible. The mass was cooled and extracted repeatedly with petroleum ether ($50-60^{\circ}$). The white crystalline residue left on evaporation was again crystallised until the m.p. rose to 69° . It was obtained as sandy crystals, m.p. 69° , yield 1.2 g. (Found : Br, 22.3. $C_{16}H_{22}O_3Br$ requires Br, 22.8 per cent).

The white residue left on evaporation of the mother-liquor was again digested with glacial acetic acid and hydrobromic acid when a further quantity of the bromo-acid was obtained.

Ethyl 16-Acetoxy-10-ketopalmitate (VI).—The bromo-acid (2 g.) was refluxed for 24 hours with 1.5 g. of freshly fused potassium acetate and glacial acetic acid (15 c.c.) over a free flame. Potassium bromide separated and was filtered off and acetic acid was removed under water pump. The dark residue was poured into water when flaky crystals separated. They were collected, dried and directly esterified by alcohol and gaseous hydrochloric acid. After pouring into water and washing with sodium bicarbonate, the residue was distilled in vacuum when the keto-ester distilled and solidified as flakes, b.p. $219-20^{\circ}/3$ mm., m.p. $54-55^{\circ}$. (Found : C, 67.9 ; H, 10.6. $C_{20}H_{30}O_5$ requires C, 67.4 ; H, 10.1 per cent)

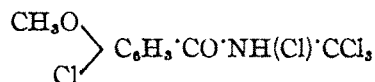
ON CHLORALAMIDES. THE REACTION OF PHOSPHORUS PENTACHLORIDE ON CHLORAL-CHLOROSALICYL- AMIDES AND THEIR METHYL ETHERS, AND THE REACTIVITY OF THE CHLORINE ATOM.

BY (LATE) N. W. HIRWE AND K. N. RANA.

The methyl ethers of chloral-chlorosalicylamides have been reacted with phosphorus pentachloride and the corresponding α -chloro compounds have been isolated.

The α -chlorine atom is very reactive and has been found to react with water, alcohols, amines, phenols and organic acids, yielding related derivatives.

Feist, Nissen and Stadler (*Ber.*, 1914, **47**, 1173) found that the α -hydroxyl group in chloral-aminonia derivatives, $R'CO \cdot NH \cdot CH(OH) \cdot CCl_3$, is replaced by halogen by phosphorus halides. Chloral-chlorosalicylamides (*Ber.*, 1939, **72**, 1346) gave no definite products owing to the presence of free phenolic group (*cf.* Anschütz, *Ber.*, 1897, **30**, 221; Cohen, "Organic Chemistry," 1931, Vol. I, p. 408). When this group is methylated definite α -chlorocompounds, *e.g.*,



are isolated.

The α -chlorine atom is far more reactive than the α -hydroxyl group (*cf.* Hirwe and Rana, *J. Univ. Bombay*, 1938, **7**, iii, 174). It reacts with water regenerating the parent compound and reacts with alcohols, amines, phenols and organic acids yielding the corresponding derivatives. The reaction is easier and quicker with alcohols and bases than with phenols and acids.

The reactivity of α -chloro compounds seems to depend on the position and number of the chlorine atoms in the nucleus. They are slowly converted by atmospheric moisture into the original compounds. Thus, they could be only crystallised from dry, inert solvents and have to be preserved in a desiccator.

EXPERIMENTAL.

α -Chlorochloral-5-chloro-2-methoxybenzamide.—A mixture of chloral-5-chloro-2-methoxybenzamide (7 g.) and phosphorus pentachloride (5 g.) was

heated on a water-bath, out of contact with atmospheric moisture, until the evolution of hydrogen chloride ceased and a clear yellow liquid resulted. When it was poured on to crushed ice after cooling, a sticky mass separated which gradually solidified after being triturated with ice-water. The solid was filtered off, washed with ice-water and dried in a desiccator. The compound, which is soluble in acetone, benzene and toluene, crystallised from dry acetone in colourless plates, m.p. $144-45^{\circ}$, yield 7 g. It gave no colouration with ferric chloride. It developed slightly violet tinge on long standing even when protected from moist air. (Found : N, 4.09 ; Cl, 50.64. $C_{10}H_8O_2NCl_3$ requires N, 3.98 ; Cl, 50.51 per cent).

α -Methoxychloral-5-chloro-2-methoxybenzamide.— α -Chlorochloral-5-chloro-2-methoxybenzamide (2 g.) was heated under reflux, protected from moisture, with methanol (20 c.c.) on a water-bath for about 2 hours, when it completely went into solution. On cooling, white cubes separated which had m.p. $131-32^{\circ}$ after recrystallisation from methanol. It gave no colouration with ferric chloride and showed no depression of m.p. on admixture with a pure sample of α -methoxychloral-5-chloro-2-methoxybenzamide (Hirwe and Rana, *loc. cit.*). (Found : Cl, 40.69. $C_{11}H_{11}O_3NCl_4$ requires Cl, 40.93 per cent).

α -Ethoxychloral-5-chloro-2-methoxybenzamide was prepared as described above, absolute alcohol replacing methanol. The compound crystallised from alcohol in white prisms, m.p. $137-38^{\circ}$ and gave no colouration with ferric chloride. (Found : Cl, 39.14. $C_{12}H_{13}O_3NCl_4$ requires Cl, 39.33 per cent).

α -Anilinochloral-5-chloro-2-methoxybenzamide.— α -Chlorochloral-5-chloro-2-methoxybenzamide (2 g.) was heated with aniline (5 c.c.) on a water-bath for 2 hours. After standing overnight at room temperature the mixture gave a pasty mass on pouring into water, which solidified when triturated with dilute hydrochloric acid. The compound crystallised from alcohol in white silky needles, m.p. $152-53^{\circ}$. It gave no ferric chloride reaction. (Found : N, 6.74 ; Cl, 34.84. $C_{16}H_{14}O_2N_2Cl_4$ requires N, 6.86 ; Cl, 34.80 per cent).

α -o-, m- and p-Toluidinochloral-5-chloro-2-methoxybenzamides were similarly prepared. These compounds gave no colouration with ferric chloride.

The *o*-toluidino derivative crystallised from alcohol in colourless, thick needles which slightly darkened on long exposure, m.p. $148-49^{\circ}$. (Found : Cl, 33.57. $C_{17}H_{16}O_2N_2Cl_4$ requires Cl, 33.65 per cent).

The *m*-toluidino derivative crystallised from alcohol in colourless flakes which also darkened on exposure, m.p. 153-54°. (Found : Cl, 33.98. $C_{17}H_{16}O_2N_2Cl_4$ requires Cl, 33.65 per cent).

The *p*-toluidino derivative crystallised in white needles from warm alcohol in which it is difficultly soluble, m. p. 169-70°. (Found : Cl, 33.67. $C_{17}H_{16}O_2N_2Cl_4$ requires Cl, 33.65 per cent).

α-Phenoxychloral-5-chloro-2-methoxybenzamide.—*α*-Chlorochloral-5-chloro-2-methoxybenzamide (2 g.) was heated with phenol (2 g.) on a water-bath for 4 hours protected from moisture. On pouring the clear mixture into ice-water an oil separated which was washed several times with water. It immediately solidified in contact with alcohol. The compound crystallised from alcohol in white, shining prisms, m.p. 194-95°. It gave no ferric reaction. (Found : Cl, 34.82. $C_{16}H_{13}O_3NCl_4$ requires Cl, 34.72 per cent).

α-Benzoyloxychloral-5-chloro-2-methoxybenzamide.—A finely powdered mixture of *α*-chlorochloral-5-chloro-2-methoxybenzamide (2 g.) and benzoic acid (2 g.), suspended in dry benzene, was heated under reflux, protected from moisture, on a water-bath for 2 hours. On concentrating the solution, a pasty mass was obtained which was treated with hot water to remove the excess of benzoic acid. The paste ultimately solidified on keeping in contact with dilute sodium bicarbonate solution for a few hours. The solid crystallised from alcohol in white small plates, m.p. 133-35° and gave no colouration with ferric chloride. (Found : Cl, 32.70. $C_{17}H_{13}O_4NCl_4$ requires Cl, 32.49 per cent).

α-Chlorochloral-3 : 5-dichloro-2-methoxybenzamide.—Chloral-3 : 5-dichloro-2-methoxybenzamide gave the substance on treatment with phosphorus pentachloride in the usual manner. The compound, which is soluble in acetone, benzene and toluene, crystallised from dry benzene in colourless, rectangular plates, m.p. 89-91°, yield 7.5 g. There was a fall of about 3-4° in the m.p. after about a week. It darkened on exposure and gave no colouration with ferric chloride. (Found : Cl, 55.09. $C_{16}H_7O_2NCl_6$ requires Cl, 55.18 per cent).

α-Methoxychloral-3 : 5-dichloro-2-methoxybenzamide.—*α*-Chlorochloral-3 : 5-dichloro-2-methoxybenzamide and methanol gave the substance when reacted in the usual manner. The compound crystallised from methanol in white prisms, m.p. 102-3°, and gave no colouration with ferric chloride. It showed no depression on admixture with an authentic specimen of *α*-methoxychloral-3 : 5-dichloro-2-methoxybenzamide (Hirwe and Rana, *loc. cit.*). (Found : Cl, 46.30. $C_{11}H_{10}O_3NCl_6$ requires Cl, 46.52 per cent).

α-Anilinochloral-3 : 5-dichloro-2-methoxybenzamide, prepared as above, crystallised from alcohol in white, shining needles, m.p. 147-48°, and gave

no colouration with ferric chloride. (Found : Cl, 40'24. $C_{16}H_{13}O_2N_2Cl_5$ requires Cl, 40'12 per cent).

o-*o*-, *m*- and *p*-Toluidinochloral-3 : 5-dichloro-2-methoxybenzamides.— The *o*-toluidino derivative, prepared in the usual manner, crystallised from alcohol in colourless, shining needles, m.p. 153-54°. (Found : Cl, 38'67. $C_{17}H_{15}O_2N_2Cl_5$ requires Cl, 38'88 per cent).

The *m*-toluidino derivative crystallised from alcohol in colourless, shining needles which, however, slightly darkened on long exposure, m.p. 146-47°. (Found : Cl, 38'75. $C_{17}H_{15}O_2N_2Cl_5$ requires Cl, 38'88 per cent).

The *p*-toluidino derivative crystallised from alcohol in white needles, m.p. 145-46°. (Found : Cl, 38'57. $C_{17}H_{15}O_2N_2Cl_5$ requires Cl, 38'88 per cent). No ferric reaction was given by any of these compounds.

o-Phenoxychloral-3 : 5-dichloro-2-methoxybenzamide was prepared from *o*-chlorochloral-3 : 5-dichloro-2-methoxybenzamide (2 g.) and phenol (2 g.). The product crystallised from alcohol in white minute needles, m.p. 125-26°, and gave no colouration with ferric chloride. (Found : Cl, 40'19. $C_{16}H_{12}O_3NCl_5$ requires Cl, 40'02 per cent).

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Received September 29, 1939

MECHANISM OF THE MICROBIOLOGICAL OXIDATION OF AMMONIA. PART II.

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The inhibitory action of capillary active substances like urethanes, nitriles, alcohols and ketones on the microbiological oxidation of ammonia has been studied by following Erlenmeyer's technique. The action of cyanides has also been studied. These substances have been found to inhibit the respiration of the bacteria for some time after which, however, nitrite begins to appear, the period of inhibition is found to increase generally with the increase in concentration of the narcotic. These experiments lead to the conclusion that the oxidation of ammonia is a surface catalytic reaction taking place at certain active centres on the surface of the bacteria. ✓

In a previous publication (*J. Indian Chem. Soc.*, 1938, **14**, 598) the question of the formation of intermediate products in the microbiological oxidation of ammonia was discussed. Our experiments showed that hydroxylamine and hyponitrite could not be detected in the process of oxidation of ammonia to nitrite.

To elucidate the mechanism of oxidation of ammonia by the bacteria we have studied the action of capillary active substances like the alcohols, the ketones and the nitriles and that of specific narcotics like potassium cyanide and hydrocyanic acid on the course of this oxidation. Meyerhof used the familiar Barcroft manometer technique for a similar study, but we made a new departure, in that we used the Erlenmeyer technique. In the first method Meyerhof followed the course of the reaction only during a small interval of time by noting the absorption of oxygen in the manometer. On the other hand, following the Erlenmeyer's technique we observed the reaction for a long period of several days, estimating the concentration of nitrite formed at intervals. Very interesting results have been obtained, which are recorded in the following pages.

EXPERIMENTAL.

The method of culture of the bacteria was the same as described in Part I.

Effect of Potassium Cyanide.—100 ml portions of the culture medium were introduced into sterilised Erlenmeyer flasks (500 ml.) and 1 g. of magnesium carbonate was added to each flask. The enriched culture (1 ml.) of the nitrite forming bacteria was pipetted by means of a sterile pipette into these flasks. Varying volumes of potassium cyanide solution were

then measured out into these flasks to give different concentrations. The total volume in each flask was made up to 200 ml. by the addition of the requisite quantity of sterile distilled water. The flasks were plugged with sterile cotton and incubated at 30°.

The amount of nitrite nitrogen in mg. per litre in each flask was estimated from time to time and the following table shows the results obtained. Figures in the tables refer to mg. of nitrite-nitrogen per litre.

TABLE I.

Influence of potassium cyanide.

Incubated for	Concentration of potassium cyanide					
	Nil. (Control.)	M/40,000	M/4,000.	M/1,000	M/250.	M/50.
0 days	2.4	2.0	2.2	2.2	2.2	2.0
18	2.2	2.2	2.2	2.2	2.2	2.8
30	4.5	2.0	2.4	2.2	2.2	2.4
41	23.3	2.2	2.2	2.1	2.3	2.2
46	41.2	2.1	2.2	2.1	2.3	2.2
54	82.9	2.3	2.2	2.2	2.2	2.1
62	112.0	6.9	3.0	2.3	2.2	2.1
69	164.0	28.0	8.0	2.6	2.5	2.2
76	—	70.9	36.8	3.3	3.3	2.2
88	—	112.0	52.8	44.4	5.1	2.4
99	—	—	80.0	50.0	26.2	2.0
107	—	—	—	65.9	40.0	2.0

TABLE II.

Influence of hydrocyanic acid.

Incubated for	Concentration of hydrocyanic acid.				
	Nil. (Control.)	M/40,000.	M/10,000	M/2,500.	M/500.
0 days	2.4	2.0	2.0	2.2	2.0
18	2.2	2.0	2.2	2.2	2.0
30	4.5	2.1	2.4	2.5	2.4
41	23.3	2.2	2.3	2.6	2.3
46	41.2	2.2	2.4	2.6	2.5
54	82.9	2.4	2.4	2.5	2.5
62	112.0	3.4	4.2	3.9	2.4
69	164.0	19.5	19.5	23.1	2.8
76	—	52.8	56.0	57.4	5.3
88	—	97.5	88.2	114.2	22.4
99	—	—	—	—	46.7
107	—	—	—	—	59.4

From the above two tables it appears that cyanides inhibit the growth and respiration of nitrite forming bacteria even at as low a concentration as $M/40,000$. In the control flask nitrite begins to form in thirty days after inoculation and steadily increases thereafter, while in the flasks containing cyanide, nitrite begins to form only after a long incubation period, the minimum period being about 62 days. The length of this period increases with increasing concentration of the cyanide. Cyanide appears to be destroyed during this inhibition and when the cyanide disappears completely, the growth and respiration of the bacteria begins leading to the formation of nitrite. A steady increase in the amount of nitrite formed is observed from this time.

A very interesting point emerges from this investigation. Even in these flasks, where the concentration of potassium cyanide is initially as high as $M/50$, the growth and respiration of bacteria begins and progresses as evidenced by the increasing accumulation of nitrite, though this happens after a long time. This shows that this narcotic effect of cyanide is reversible; the cyanide does not permanently kill the bacteria but exerts only a temporary inhibition on the respiration. During the long period of inhibition the cyanide disappears and then the respiration and growth of bacteria start. In the presence of cyanide, the bacteria are in some sort of quiescent state but are not killed. It would be an interesting work for bacteriologists to define exactly what this quiescent inactive state is. We express the hope that these results may be of considerable importance in connection with the poisonous action of cyanides on higher animals.

Gopala Rao and Pandalai (*Archiv Mikrobiol.*, 1936, 7, 32) obtained similar results with potassium ferrocyanide and ferricyanide. They found that the narcotic effect can be demonstrated even in actively growing cultures. Now we have found that the appearance of nitrite after a long time in flasks to which cyanide was added at the start is not due to the bacteria getting acclimatised to the narcotic; for if cyanide is again added after a considerable accumulation of nitrite takes place, the respiration of the bacteria is again inhibited. This was confirmed in another way. Inoculations from cultures grown in presence of cyanide were made into fresh media containing cyanide in order to study whether bacteria, which remained inactive in the presence of cyanide for a long time and later resumed normal activity, can resist the action of fresh amounts of the poison. These experiments showed that such cultures were as much inhibited by the fresh cyanide as any other, while they are capable of growing and forming nitrite in a medium free from cyanide.

Effect of Capillary Active Substances.—In the following tables the effect of different capillary active substances were studied in the same manner as in the case of potassium cyanide.

TABLE III.

Influence of methylurethane.

Concentration of methylurethane.

Incubated for	Nil. (Control.)	M/10.	M/20	M/50
9 days	2.3	2.3	2.3	2.2
15	3.7	2.1	2.1	2.1
22	22.9	2.1	2.0	2.1
30	40.0	2.0	2.1	2.0
37	97.4	2.0	2.0	2.2
42	112.0	2.2	2.0	2.0
58	156.5	2.0	2.1	2.3
69	172.3	2.0	2.1	2.3

TABLE IV.

Influence of phenylurethane.

Concentration of phenylurethane.

Incubated for	Nil. (Control.)	M/500.	M/1,000	M/2,000.
9 days	2.3	2.0	2.3	2.3
15	3.7	2.0	2.3	2.6
22	22.9	1.9	2.1	3.4
30	40.0	1.9	2.1	8.0
37	97.4	2.1	2.1	10.0
58	156.5	2.0	2.3	19.5
69	172.3	2.1	2.3	51.6

TABLE V.

Influence of acetonitrile.

Concentration of acetonitrile.

Incubated for	Nil. (Control.)	M/50.	M/100.	M/500.	M/1,000.
10 days	1.8	1.9	1.8	1.9	1.9
16	2.3	1.9	1.9	2.0	2.0
23	3.0	1.9	2.0	2.2	2.5
30	19.1	1.9	2.0	2.0	5.9
37	31.6	2.1	2.1	2.4	9.4
42	46.3	2.0	2.0	2.4	41.8
58	70.0	2.0	2.0	3.2	103.0
69	102.6	2.0	2.0	21.2	110.0

TABLE VI.

Influence of propionitrile.

Incubated for	Concentration of propionitrile.			
	Nil (Control)	M/100	M/500.	M/1,000.
10 days	1.8	1.9	1.9	1.8
16	2.3	1.9	1.9	1.8
23	3.0	2.1	2.0	2.1
30	19.1	2.1	2.0	2.1
37	31.6	2.2	2.1	3.4
42	46.3	2.0	2.0	4.2
58	70.0	2.0	2.0	32.9
69	102.6	2.3	2.2	80.0

TABLE VII.

Influence of butyronitrile.

Incubated for	Concentration of butyronitrile.			
	Nil. (Control.)	M/1,000.	M/5,000.	M/10,000.
0 days	1.8	1.8	1.8	1.8
23	4.9	1.8	38.8	46.6
29	25.5	6.0	83.6	82.9
36	39.4	28.0	113.0	100.0
43	72.8	37.3	118.0	154.0
52	118.0	112.0	195.0	195.0

TABLE VIII.

Influence of benzonitrile.

Incubated for	Concentration of benzonitrile.			
	Nil. (Control.)	M/500.	M/1,000.	M/5,000.
0 days	1.8	1.8	1.9	1.8
16	2.3	1.8	6.4	3.0
23	3.0	20.0	22.0	10.7
30	19.1	33.5	36.6	27.1
37	31.6	94.0	96.5	67.5

It has been found that there is an acceleration even at a concentration of $M/10,000$ benzonitrile. Previously, Gopala Rao and Pandalai (*loc. cit.*) observed that benzonitrile markedly inhibits respiration and growth of the bacteria at concentrations ranging from $M/10$ to $M/100$.

TABLE IX.

Influence of methyl alcohol.

Concentration of methyl alcohol.

Incubated for	Nil Control. (1)	M/10	M/20	M/40.	M/100.	Nil Control. (2)
0 days.	2.3	2.0	2.1	2.1	2.1	2.3
12	3.6	—	—	—	—	4.5
19	9.7	—	—	—	—	10.9
26	32.9	—	—	—	7.5	27.6
35	43.1	—	2.2	—	—	48.7
46	115.4	5.3	11.2	5.3	47.3	107.9
58	130.2	24.3	21.1	22.8	101.8	—
64	—	35	43.1	42.4	—	—
84	—	—	78.3	80	—	—

TABLE X.

Influence of ethyl alcohol.

Concentration of ethyl alcohol.

Incubated for	M/10.	M/20.	M/40.	M/100.	Nil Control.
0 days	—	—	—	—	2.7
12	—	—	—	—	4.5
19	—	—	—	—	10.9
26	—	—	—	—	27.6
35	—	—	—	—	48.7
46	—	—	—	—	107.9
58	—	—	4.9	4.0	—
64	—	4.0	22.4	23.5	—
84	2.4	50.0	50.0	74.7	—
99	41.5	74.7	74.7	93.8	149.3

TABLE XI.

Influence of octyl alcohol.

Incubated for	Concentration of ethyl alcohol.			
	Nil. (Control.)	M/250.	M/500.	M/1,000.
0 days	2.7	1.9	2.1	1.9
12	4.5	2.1	2.3	2.1
19	10.9	1.9	2.3	1.6
26	27.6	2.2	2.0	—
35	48.7	2.2	—	—
46	107.9	2.4	—	—
58	—	1.9	—	—
64	—	1.9	—	—
84	—	1.8	0.7	0.5
99	149.3	1.7	19.8	30.6
108	—	1.5	25.2	45.2

It has been found that propyl alcohol at a concentration of $M/250$ slightly accelerates, while even at a concentration of $M/50$ there is no marked inhibition of the respiration (results not given).

TABLE XII.

Influence of acetone.

Concentration of acetone.

Incubated for	Nil. (Control.)	M/100.	M/50.	M/20.
0 days	2.3	2.2	2.0	2.0
7	3.5	3.2	2.8	2.6
23	15.1	23.3	14.0	3.1
33	46.6	46.6	25.0	6.2

TABLE XIII.

Influence of methyl ethyl ketone.

Concentration of methyl ethyl ketone.

Incubated for	Nil. (Control.)	M/500.	M/100.	M/50.
0 days	2.4	2.2	1.9	1.9
12	7.0	8.6	8.4	4.3
19	34.2	49.1	35.0	30.3
28	74.7	112.0	112.0	70.0

TABLE XIV.

Influence of methyl hexyl ketone.

Incubated for	Concentration of ketone.			
	Nil (Control.)	M/1,000.	M/500	M/100
0 days	1.7	1.7	1.4	1.8
18	9.9	1.0	1.3	1.7
28	41.0	9.6	8.0	1.8
34	76.7	32.0	32.4	1.6
54	158.0	97.1	93.4	2.2

TABLE XV.

Influence of benzophenone.

Incubated for	Concentration of benzophenone			
	Nil (Control.)	M/4,000	M/2,000.	M/1,000.
7 days	1.7	1.5	1.5	1.4
18	9.9	1.6	1.5	1.6
28	41.0	1.9	1.7	1.8
34	76.7	1.9	1.8	1.8
54	158.0	2.2	2.3	2.3
66	160.0	2.2	1.7	1.7

DISCUSSION.

Gopala Rao and Pandalai have shown that it is unlikely that the biological oxidation of ammonia to nitrite by the nitrite forming bacteria involves a peroxidase-peroxide system. Pandalai (*Biochem. Z.*, 1939, **300**, 122) could not detect any dehydrogenase activity in the nitrite-forming bacteria.

Recent work of Quastel (*Biochem. J.*, 1926, **20**, 166), Quastel and Wooldridge (*ibid.*, 1927, **21**, 148, 1224; 1928, **22**, 689) and of others has shown that a suspension of bacteria behaves in a manner identical with any colloid system possessing catalytic properties and can be investigated precisely in the same way. The fact that a suspension of resting bacteria can be regarded in a manner similar to a catalytic system is indicated by its ability to bring about reversible reactions whose equilibrium points are independent of the amount and the conditions of the organism employed. Cook and Woolf (*Biochem. J.*, 1926, **20**, 545; 1928, **22**, 474)) have demonstrated that the equilibrium point of the reversible formation of aspartic acid from fumaric acid and ammonia is the same with a number of different organisms and muscle tissue. The fact that dissimilar biological materials give the same equilibrium point is a good indication that truly catalytic systems are being investigated.

With some bacteria the range of activation seems to be very wide. *B. Coli* has been found to activate at least 56 substances so that they could reduce methylene blue in vacuum. A systematic study by Quastel of the effect of various selective poisons on *B. Coli* has revealed that the activation is associated with definite specially active patches; and that these patches have specificity or the power of discrimination between different substrates. The power of a given patch to adsorb different substrates depends on their chemical constitution, so that only a few substances are adsorbed; and of those substances which are adsorbed, only a small proportion is activated. The active patches on the cell surface vary in their capacity for activation considerably—some active centres will be capable of activating only certain types of molecules, while they are without action on other molecules. These experiments bring to light the close analogy of bacterial reactions in heterogeneous catalytic reactions occurring at the surface of metal or metal oxide catalysts. Much of the work on surface catalysis carried out within recent years has gone to show that catalytic activity occurs at particular active patches on the surface and that these active patches may vary in their capacity to adsorb reactants and activate them for chemical reaction.

Some bacteria may not have such a wide range of activation as is the case with *B. Coli*. They may be very highly specific and activate only one type of molecules. To this class belong the organisms that can bring about the oxidation of ammonia to nitrite. So far it has been found that only ammonia is activated by these bacteria.

It may be considered that as the cell grows the various synthetic processes culminating in the formation of proteins, etc., are such as to bring about the formation of active centres on the structures. The active patches are simply a property of the surface structures of the colloidal materials which make up the cell as a whole and is simply due to the conditions which obtain in the living cell, so that just that arrangement or juxtaposition of cellular material occurs with the formation of active centres. The centres may be regarded as points or regions of instability, places where a loose fitting of some of the molecules constituting the cellular aggregates occurs. The actual magnitude of the colloidal structures containing the centre is not of great significance. It would be expected that the smaller the structure, the less chance is there of its possessing a number of different centres, and hence of possessing an extensive range of activation. This may explain the exclusively specific nature of the nitrifying organisms—these organisms appear to be very elementary in their development, for they cannot utilise any of the complex organic molecules. They oxidise ammonia and the energy liberated in this exothermal reaction is partly utilised for their life

activities and partly for the chemosynthesis of carbohydrates from the carbon dioxide of the atmosphere. Other organisms of this type which are only capable of acting on inorganic metabolites such as the hydrogen bacteria, the nitrite oxidising bacteria, etc. are highly specific and act only on one substrate; whereas the more highly organised bacteria such as the *B. Coli*, the pneumococcus, the staphylococcus, etc. are not so exclusively specific but are active towards quite a large number of substrates. It appears that the higher an organism is placed in the scale of evolution, the wider the range of its activity.

The results presented in this paper show that the oxidation of ammonia occurs as a heterogeneous catalytic reaction at the surface of the bacterial cells. The inhibitory action of urethanes, alcohols, etc. shows that the action is not specific and that the "poisons" simply act by competing with the substrate for the space available for adsorption at the active centres of cellular surface. The inhibition by these substances is marked only at sufficiently high concentrations. It will also be observed that in the same series of compounds, the concentration required for marked inhibition is lower in the case of a compound of a higher molecular weight than in the case of one of low molecular weight. Thus in the case of ethyl alcohol marked inhibition is obtained at a concentration of $M/10$, whereas with the octyl alcohol a similar result is obtained even at a concentration of $M/500$; acetone produces marked inhibition at $M/20$, methyl hexyl ketone at $M/100$ and benzophenone at $M/4,000$. These results could be explained on the supposition that adsorbability and inhibitory capacity run parallel to one another. It is well known from Traube's rule that in a homologous series adsorbability of a compound increases as the molecular weight is increased.

Curiously enough it is noticed that butyronitrile and benzonitrile at low concentrations of the order of $M/5,000$ — $M/1,000$ accelerate the reaction instead of retarding it; the inhibitory action of benzonitrile is manifest only at higher concentrations, say $M/100$.

While the inhibitory action of the urethanes, alcohols, ketones, etc. is manifest only at relatively high concentrations, potassium cyanide and hydrocyanic acid completely inhibit the growth and respiration of the bacteria at extremely low concentrations of the order of $M/40,000$. This result shows that activity of the centres is very likely due to a complex containing a heavy metal like iron. The cyanide acts by selectively poisoning these iron-rich centres.

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